Fungal Biology

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Recent Advancement in White Biotechnology Through Fungi

Volume 1: Diversity and Enzymes Perspectives

Springer

Fungal Biology

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About the Series

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with *living and non-living* is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification and are now being extended across fungal phyla. The *majorities of fungi are multicellular eukaryotic systems* and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

More information about this series at http://www.springer.com/series/11224

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Foreword

White biotechnology, also known as industrial biotechnology, refers to the use of living cells and/or their enzymes to create industrial products that are more easily degradable, require less energy, create less waste during production and sometimes perform better than the products created using traditional chemical processes. In the twenty-first century, technology was developed to harness fungi to protect human health (through antibiotics, antimicrobial, immunosuppressive agents, value-added products, etc.), which led to industrial-scale production of enzymes, alkaloids, detergents, acids and biosurfactants. During the last decade, considerable progress has been made in white biotechnology research, and further major scientific and technological breakthroughs are expected in the near future. The first large-scale industrial applications of modern biotechnology have been made in the areas of food and animal feed production (agricultural/green biotechnology) and of pharmaceuticals (medical/red biotechnology). In contrast, the production of bioactive compounds through fermentation or enzymatic conversion is known as industrial or white biotechnology. The fungi that are ubiquitous in nature have been isolated from diverse habitats including extreme environments (high temperature, low temperature, salinity, drought, radiation, pressure and pH) and may be associated with plants as epiphytic, endophytic and rhizospheric. Fungal strains are beneficial as well as harmful for human beings. The beneficial fungal strains may play an important role in agriculture, industry and medical sectors. The beneficial fungi play a significance role in plant growth promotion and soil fertility using both direct (solubilization of phosphorus, potassium and zinc; production of indole acetic acid, gibberellic acid, cytokinin and siderophores) and indirect (production of hydrolytic enzymes, siderophores, ammonia, hydrogen cyanides and antibiotics) mechanisms of plant growth promotion for sustainable agriculture. The fungal strains and their products (enzymes, bioactive compounds and secondary metabolites) are very useful for industry, e.g. the discovery of penicillin from *Penicillium chrysogenum* is a milestone in the development of white biotechnology into a fully fledged global industrial technology. Since then, white biotechnology has steadily developed and now plays a key role in several industrial sectors, providing both high-valued nutraceuticals and pharmaceutical products. Fungal strains and bioactive compounds also play important role in the environmental cleaning.

The present volume on *Recent Advancement in White Biotechnology Through Fungi Vol. 1: Diversity and Enzymes Perspectives* is a very timely publication, which provides state-of-the-art information in the area of white biotechnology, broadly involving fungal-based innovations and applications. This volume comprises 17 chapters. Chapter 1 by Rana et al. describes biodiversity of endophytic fungi from diverse plants, producing wide groups of extracellular hydrolytic enzymes and secondary metabolites for plant growth and soil health, environment bioremediation and bioactive compounds. Chapter 2, presented by Pattnaik and Busi, highlights the interaction of rhizospheric fungi with plants and their potential applications in different fields including agriculture, industrial, pharmaceuticals and biomedical sectors. Chapter 3 by Sharma et al. describes the biodiversity of a ubiquitous fungus, *Trichoderma*, from diverse sources and its applications in the industry as producer of bioactive compounds and extracellular hydrolytic enzymes and in the agriculture as plant growth prompter and biocontrol agents. Chapter 4 by Abdel-Azeem et al. highlights the potential of fungus *Aspergillus*, its biodiversity, ecological significance and industrial applications. In Chapter 5, Pandey et al. describe the mycorrhizal fungi and their biodiversity, ecological significance and industrial applications. Chapter 6 by Abdel-Azeem et al. gives an overview of the studies aimed at the investigation of *Fusarium* biodiversity in a wide variety of different ecological habitats, ecological significances and potential industrial applications. Chapter 7, authored by Naik et al., deals with the new perspectives of industrially important enzymes from endophytic fungi. The enzymes from endophytic fungi have significant potential applications in various industries dealing with chemicals, fuels, food, brewery and wine, animal feed textile, laundry, agriculture, pulp and paper. In Chap. 8, Halder and colleagues emphasize the biosynthesis of fungal chitinolytic enzymes and their potential biotechnological applications in industry and allied sectors. Salwan and Sharma describe the tool for white biotechnology by extremophilic fungal protease production and their applications in Chap. 9. Mandal and Banerjee explain the protease enzymes originating from diverse endophytic fungi and industrial applications in Chap. 10. The most important applications of lipase in pharmaceuticals, pulp and paper, chemicals, textile industries, food processing and biodiesel production have been described by Pérez et al. in Chap. 11. Chapter 12 by Singh et al. describes fungal xylanases, their sources, types and potential biotechnological applications. Susana Rodríguez-Couto presents an overview of fungal laccase, a versatile enzyme for biotechnological applications, in Chap. 13. Karnwal et al. discuss enzymes from different groups of fungi for the textile industry in Chap. 14. Ecological and industrial perspectives of marine fungal white biotechnology are discussed in Chap. 15 by Vala et al. Chapter 16 by Berde et al. highlights the discovery of new extremophilic enzymes from diverse fungal communities and their potential applications in agricultural, industrial, pharmaceutical and allied sectors. Finally, the overall status of fungal white biotechnology is described in Chap. 17 by Meena et al. as the global scenario of fungal white biotechnology in the past, present and future.

Overall, great efforts have been carried out by Dr. Ajar Nath Yadav, his editorial team and scientists from different countries to compile this book as a highly unique, up-to-date source on fungal white biotechnology for students, researchers, scientists and academics. I hope that the readers will find this book highly useful and interesting during their pursuit on fungal biotechnology.

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Dr. H. S. Dhaliwal is presently the vice chancellor of Eternal University, Baru Sahib, Himachal Pradesh, India. Dr. Dhaliwal holds PhD in genetics from the University of California, Riverside, USA (1975). He has 40 years of research, teaching and administrative experience in various capacities. Dr. Dhaliwal is a professor of biotechnology at Eternal University, Baru Sahib, from 2011 to date. He worked as the professor of biotechnology at IIT, Roorkee (2003–2011); founding director of Biotechnology Centre, Punjab Agricultural University, Ludhiana (1992–2003); senior scientist and wheat breeder-cum-director at PAU's Regional Research Station, Gurdaspur (1979–1990); research fellow FMI, Basel Switzerland (1976–1979); and D.F. Jones postdoctoral fellow, University of California, Riverside, USA (1975–1976). Dr. Dhaliwal was elected as fellow, National Academy of Agricultural Sciences, India, (1992); worked as visiting professor, Department of Plant Pathology, Kansas State University, Kansas, USA, (1989); and was a senior research fellow, CIMMYT, Mexico, (1987). He has many national and international awards to his name such as Pesticide India Award from Mycology and Plant Pathology Society of India (1988) and Cash Award from the Federation of Indian Chambers of Commerce and Industry (FICCI) in 1985. He has to his credit more than 400 publications including 250 research papers, 12 reviews, 15 chapters contributed to books, 105 papers presented in meetings, conferences and abstracted, 18 popular articles and 2 books/bulletins/manuals. His important research contributions are the following: identification of new species of wild diploid wheat *Triticumu rartu* and gathered evidences to implicate *T. urartu* as one of the parents of polyploid wheat; team leader in the development of seven wheat varieties, viz., PBW 54, PBW 120, PBW 138, PBW 175, PBW 222, PBW 226 and PBW 299 approved for cultivation in Punjab and North Western Plain Zone of India; molecular marker-assisted pyramiding of bacterial blight resistance genes *Xa*21 and *Xa*13; and the green revolution semi-dwarfing gene *sd*1 in Dehraduni basmati and developed elite wheat lines biofortified for grain rich in iron and zinc through wide hybridization with related non-progenitor wild wheat species and molecular breeding. Dr. Dhaliwal made a significant contribution to the development of life and epidemiology life cycle of *Tilletia indica* fungus, the causal organism of Karnal bunt disease of wheat and development of Karnal bunt resistance wheat cultivar. Dr. Dhaliwal is a member of several task forces and committees of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi; chairman, Project Monitoring Committee for Wheat Quality Breeding, Department of Biotechnology, Ministry of Science and Technology, Government of India (2007–2010); chairman of the Project Monitoring Committee in “Agri-biotechnology” of the Department of Biotechnology, Govt. of India, New Delhi (2014–2016); and presently, member of newly constituted Expert Committee for DBT-UDSC Partnership Centre on Genetic Manipulation of Crop Plants at UDSC, New Delhi (2016 onwards).

Preface

White biotechnology, or industrial biotechnology, is drawing much attention as a solution to produce energy, chemicals and other materials from renewable resources. White biotechnology works by marshalling living cells into micro-factories by using biomass as feedstocks. The fungi are used to synthesize functional bioactive compounds, hydrolytic enzymes, and compounds for plant growth promotion and biocontrol agents for the potential biotechnological applications in agriculture, medicine, industry, pharmaceuticals, and allied sectors. White fungal biotechnology is an emerging field in the science arena that supports the revelation of novel and vital biotechnological components. Fungi uses are divided in five major economically important fields: drug manufacturing, food and dietary, environmental, agriculture and biotechnology. The fungi *Aspergillus*, *Bipolaris*, *Cordyceps*, *Fusarium*, *Gaeumannomyces*, *Myceliophthora*, *Penicillium*, *Phoma*, *Piriformospora*, *Pleurotus*, *Trichoderma* and *Xylaria* are highly important fungal groups which can be utilized for production of different antibiotics, enzymes and peptides useful in medical and industrial fields. Secretomic analysis is one of the prominent hubs to identify secretion of enzymes, and the production can be maximized by using genetic engineering approaches in the white biotechnological field.

The present book on *Recent Advancement in White Biotechnology Through Fungi Vol. 1: Diversity and Enzymes Perspectives* covers the biodiversity of diverse groups of fungi reported from extreme environments such as temperature, salinity, drought, radiation, pressure and pH; plant associated as endophytic, epiphytic and rhizospheric; and productions of extracellular enzymes, secondary metabolites and bioactive compounds for diverse processes targeted at therapeutics, diagnostics, bioremediation, agriculture and industries. This book should be immensely useful for the biological sciences, especially to microbiologists, microbial biotechnologists, biochemists, and researchers and scientists of fungal biotechnology. We are honoured that the leading scientists with extensive, in-depth experience and expertise in fungal systems and microbial biotechnology took the time and effort to develop these outstanding chapters. Each chapter is written by internationally recognized researchers/scientists so the reader is given an up-to-date and detailed account of our knowledge of the white biotechnology and innumerable industrial applications of fungi.

We are indebted to the many people who helped to bring this book to light. The editors wish to thank Mr. Eric Stannard, Senior Editor, Botany, Springer; Dr. Vijai Kumar Gupta and Dr. Maria G. Tuohy, Series Editors, Fungal Biology Springer; and Mr. Rahul Sharma, Project Coordinator, Springer, for the generous assistance, constant support and patience in initializing the volume. Dr. Ajar Nath Yadav gives special thanks to his exquisite wife, Ms. Neelam Yadav, for her constant support and motivations in putting everything together. Dr. Yadav also gives special thanks to his esteemed friends, well-wishers, colleagues and senior faculty members of Eternal University, Baru Sahib, India.

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Chapter 1
Endophytic Fungi: Biodiversity, Ecological Significance, and Potential Industrial Applications

Kusam Lata Rana, Divjot Kour, Imran Sheikh, Anu Dhiman, Neelam Yadav, Ajar Nath Yadav, Ali A. Rastegari, Karan Singh, and Anil Kumar Saxena

Abstract Endophytic fungi are abundant and have been reported from all tissues such as roots, stems, leaves, flowers, and fruits. In recent years, research into the beneficial use of endophytic fungi has increased worldwide. In this chapter, we critically review the production of a wide range of secondary metabolites, bioactive compounds from fungal endophytes that are a potential alternative source of secondary plant metabolites and natural producers of high-demand drugs. One of the major areas in endophytic research that holds both economic and environmental potential is bioremediation. During their life span, microbes adapt fast to environmental pollutants and remediate their surrounding microenvironment. In the last two decades, bioremediation has arisen as a suitable alternative for remediating large polluted sites. Endophytic fungi producing ligninolytic enzymes have possible biotechnological applications in lignocellulosic biorefineries. This chapter highlights the recent progress that has been made in screening endophytic fungi for the

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production and commercialization of certain biologically active compounds of fungal endophytic origin.

1.1 Introduction

Microbes such as fungi, bacteria, cyanobacteria, and actinomycetes belonging to a class of plant symbionts residing within plant tissue are referred to as "endophytes" (De Bary 1866). From the germination of seeds to the development of fruits, endophytic microorganisms are associated with different parts of the plant, such as the spermosphere (in seeds), rhizosphere (roots), caulosphere (in stems), phylloplane (in leaves), anthosphere (in flowers), and laimosphere and carposphere (in fruits) (Clay and Holah 1999). To adapt to abiotic and biotic stress factors, endophytic microbes produce bioactive substances (Guo et al. 2008). The associations of endophytic microbes with plants, and in many cases their tolerance to biotic stress factors, have correlated with fungal natural products or biologically active metabolites, such as enzymes, phytohormones, nutrients, and minerals, and also enhance the resistance of the host against herbivores, insects, disease, drought, phytopathogens, and variations in temperature and salinity (Breen 1994; Brem and Leuchtmann 2001; Schulz et al. 2002). Endophytic microbes enhance the resistance of plants to abiotic stress factors such as increasing drought tolerance, high temperature, low temperature, low pH, high salinity, and the presence of heavy metals in the soil (Jalgaonwala et al. 2017). On the other hand, plants provide a protective environment for the growth and multiplication of endophytic microbes, protection from aridness, and longevity via seed transmission to the next generation of host (Khan et al. 2015). One widespread phenomenon in nature is the symbiotic association between fungus and plant.

Initial information about fungal endophytes was found during the year 1904, from endophytes isolated from the seeds of darnel ryegrass (Bezerra et al. 2012; Freeman 1904). Endophytic fungi are a diverse and useful group of microorganisms reported to colonize plants in different parts of world, such as the Arctic (Fisher et al. 1995) and Antarctic (Rosa et al. 2009), and in geothermal lands (Redman et al. 2002), deserts (Bashyal et al. 2005), oceans (Wang et al. 2006b), rainforests (Strobel 2002), mangrove swamps (Lin et al. 2008b), and coastal forests (Suryanarayanan et al. 2005). Various secondary metabolites, for instance, alkaloids, cyclohexanes, flavonoids, hydrocarbons, quinines, and terpenes, have been reported to be synthesized by fungal endophytes and have various biological properties including antimicrobial, antioxidant, antidiabetic, anticancer, antihypercholesterolemic, and antiproliferative activities and cytotoxicity, and they are used in biofuel manufacturing (Fernandes et al. 2015; Naik and Krishnamurthy 2010; Ruma et al. 2013). Endophytic fungi produce various kinds of extracellular enzymes, i.e., hydrolases, lyases, oxidoreductases, and (Traving et al. 2015). In another study, endophytic microbes producing enzymes could help to initiate the symbiotic process (Hallmann et al. 1997). Fungal endophytes have been reported to produce hydrolytic enzymes such as cellulase, lipoidase, pectinase, proteinase, and phenol oxidase so as to overcome the defense response against the host (Krishnamurthy and Naik 2017; Naik et al. 2009; Oses et al. 2006). Various organic compounds, for instance, cellulose, glucose, hemicelluloses, keratin, lignin, lipids, oligosaccharides, pectin, and proteins, have been reported to be degraded by the endophytic fungi (Kudanga and Mwenje 2005; Tomita 2003). Endophytic microbes have been reported in almost all plant studies (Suman et al. 2016; Verma et al. 2013, 2014a, 2015a). This chapter describes the biodiversity of endophytic fungi from diverse plants, producing wide groups of extracellular hydrolytic enzymes, bioactive compounds, and secondary metabolites useful for plant growth and soil health for sustainable agriculture, for environment bioremediation, and for different processes in industry.

1.2 Biodiversity and Distribution of Fungal Endophytes

Recently, a greater progress has been made in fungal endophytic research. Fungal endophytes have been found to colonize land plants everywhere on earth. They have been isolated from boreal forests, tropical climates, diverse xeric environments, extreme arctic environments, ferns, gymnosperms, and angiosperms (Mohali et al. 2005; Selim et al. 2017; Šraj-Kržič et al. 2006; Suryanarayanan et al. 2000). Endophytic fungi play an important role in protecting their host from attack by phytopathogens and also facilitate the solubilization of the macronutrients phosphorus, potassium, and zinc; the fixation of atmospheric nitrogen; and the production of various hydrolytic enzymes, ammonia, siderophore, and hydrogen cyanide (HCN) (Maheshwari 2011; Rana et al. 2016a, b, 2017; Verma et al. 2015b, c, 2016a, b).

From a review of the diverse research on endophytic fungi diversity, it can be concluded that reported fungi belong to diverse phyla including Ascomycota, Basidiomycota, and Mucoromycota (Fig. 1.1a). Figure 1.1b presents the biodiversity and abundance of endophytic fungi reported from chick pea, common pea, maize, pigeon pea, rice, soybean, tomato, and wheat. Figure 1.1c presents the relative distribution and biodiversity of endophytic fungi reported from different host plants, showing the common and host-specific endophytic fungi. Figure 1.1d is a Venn diagram showing the endophytic fungal diversity of leguminous and nonleguminous crops. There are many reports of the microbiomes as niche-specific diversity caused by diverse environmental conditions, including low temperature (Yadav 2015; Yadav et al. 2015a, b, 2016, 2017c), high temperature (Kumar et al. 2014; Sahay et al. 2017), salinity (Yadav et al. 2015c, 2018a), drought (Verma et al. 2014a, 2016b), pH (Verma et al. 2013), and multiple extreme conditions (Saxena et al. 2016; Verma et al. 2017; Yadav et al. 2015c, 2018b). Suman et al. (2016) reported niche-specific endophytic microbes from 17 different host plants. Table 1.1 presents the biodiversity of endophytic fungi reported from these diverse host plants.

Impullitti and Malvick (2013) reported fungal endophytes such as *Alternaria* sp., *Cladosporium* sp., *Davidella* sp., *Diaporthe* sp., *Epicoccum* sp., *Fusarium* sp., *Phialophora* sp., *Phoma* sp., *Phomopsis* sp., *Plectosphaerella* sp., *Trichoderma* sp.,

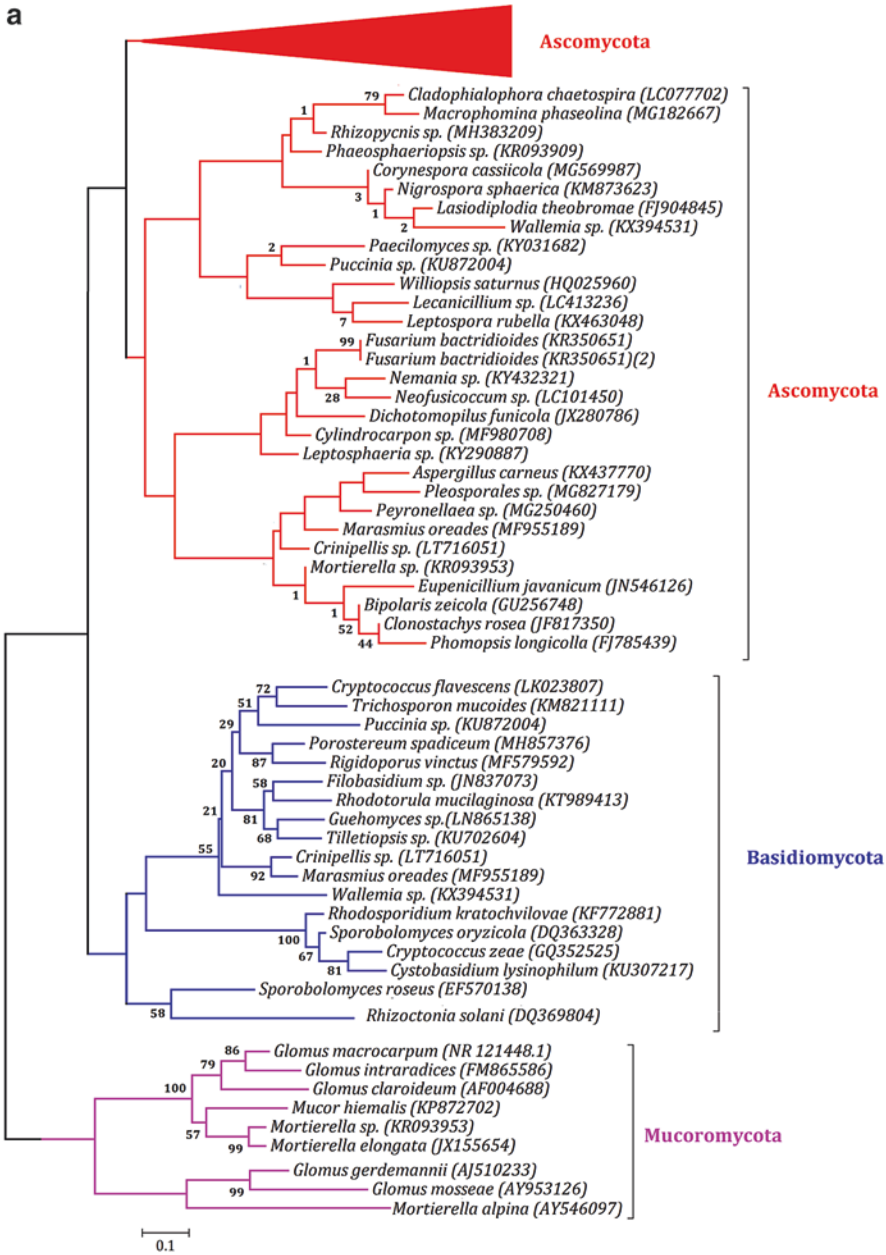

Fig. 1.1 (**a**) Phylogenetic tree shows the relationship among different groups of endophytic fungi isolated from different host plants. (**b**) Abundance of endophytic fungi belonging to diverse phyla isolated from different host plants. (**c**) Diversity and distribution of endophytic fungi of different crops. (**d**) Venn diagram showing niche-specific microbes reported from leguminous and nonleguminous crops. Wheat *(Triticum aestivum)*: (Colla et al. 2015; Comby et al. 2017; Fisher and Petrini 1992; Keyser et al. 2016; Köhl et al. 2015; Larran et al. 2002, 2007, 2018; Ofek-Lalzar et al. 2016; Sieber et al. 1988; Spagnoletti et al. 2017; Wakelin et al. 2004); rice (*Oryza sativa*): (Naik et al. 2009;

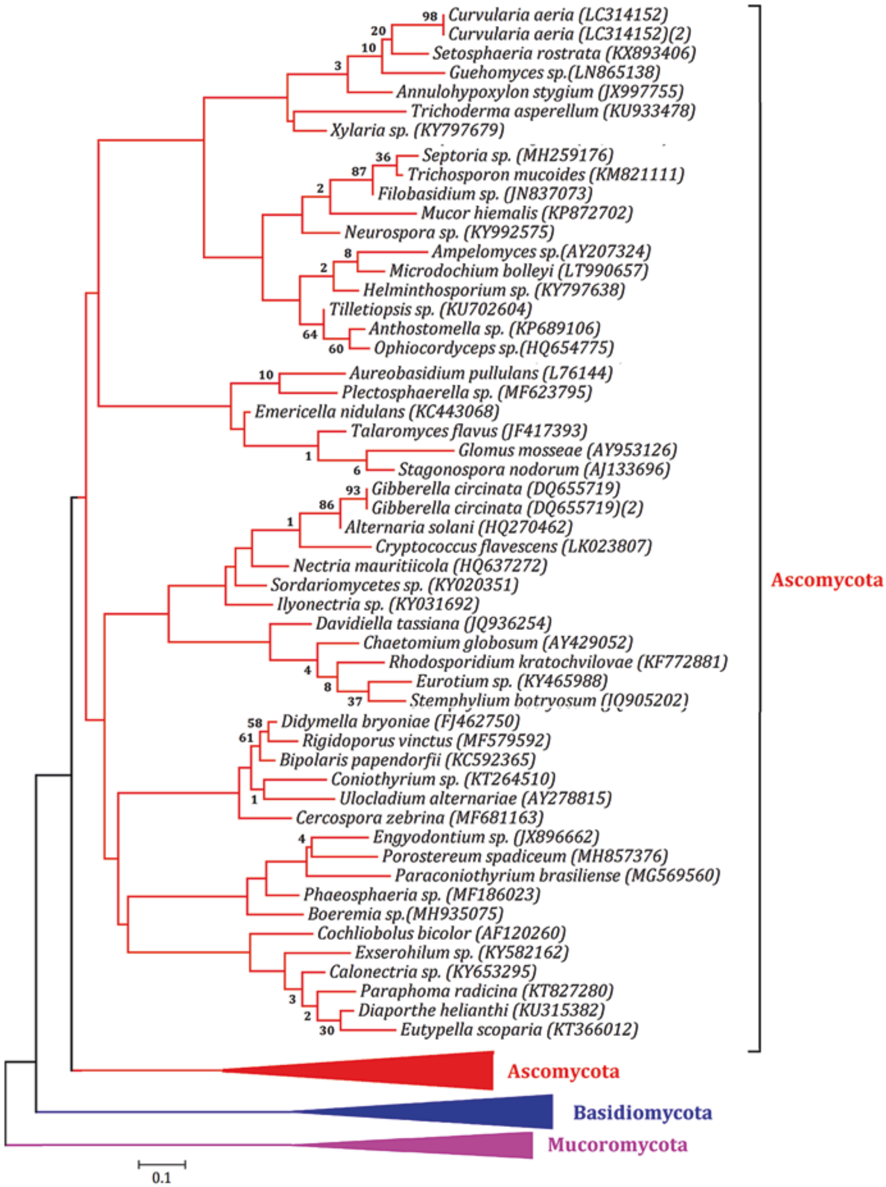

Fig. 1.1 (continued) Potshangbam et al. 2017; Tian et al. 2004; Wang et al. 2016; Yuan et al. 2010); tomato (*Solanum lycopersicum*): (Bogner et al. 2016; Chadha et al. 2015; Larran et al. 2001; Tian et al. 2014); maize (*Zea mays*): (Amin 2013; Köhl et al. 2015; Nassar et al. 2005; Pan et al. 2008; Potshangbam et al. 2017; Renuka and Ramanujam 2016; Saunders and Kohn 2008; Xing et al. 2018); chickpea (*Cicer arietinum*): (Narayan et al. 2017; Singh and Gaur 2017); soybean (*Glycine max*): (de Souza Leite et al. 2013; Fernandes et al. 2015; Hamayun et al. 2017; Impullitti and Malvick 2013; Khan et al. 2011b, 2012b; Rothen et al. 2017; Tenguria and Firodiya 2013; Yang et al. 2014, 2018; Zhao et al. 2018); common bean (*Phaseolus vulgaris*): (dos Santos et al. 2016; Gonzaga et al. 2015; Marcenaro and Valkonen 2016; Parsa et al. 2016; Pierre et al. 2016); pigeon pea (*Cajanus cajan*): (Gao et al. 2011, 2012; Zhao et al. 2012, 2013, 2014)