BIODIVERSITAS
Volume 13, Number 2, April 2012
Pages: 59-64

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d130202

Isolation and phylogenetic relationship of orchid-mycorrhiza from *Spathoglottis plicata* of Papua using mitochondrial ribosomal large subunit (mt-Ls) DNA

SUPENI SUFAATI♥, VERENA AGUSTINI, SUHARNO
Department of Biology, Faculty of Mathematics and Natural Sciences, University of Cenderawasih (UNCEN), Jayapura 99358, Papua, Indonesia. Tel. +62-967-572116. email: ♥penisufaati@yahoo.com; verena_agustini@yahoo.com, harn774@yahoo.com

Manuscript received: 26 June 2011. Revision accepted: 16 April 2012.

ABSTRACT

Sufaati S, Agustini V, Suharno. 2012. Isolation and phylogenetic relationship of orchid-mycorrhiza from Spathoglottis plicata *of Papua using mitochondrial ribosomal large subunit (mt-Ls) DNA. Biodiversitas 13: 59-64.* All terrestrial mycorrhiza have mutual symbiotic with mycorrhizal fungi in order to gain nutrient from surrounding environment. This study was done to isolate and to identify mycorrhiza orchid that associates with *Spathoglottis plicata* and were collected from Cagar Alam Pegunungan Cycloops (CAPC), Jayapura. Isolation of mycorrhizal orchid came after the modified method of Manoch and Lohsomboon (1992). The result showed that based on the morphological characteristic, there were presumably 14 isolations. However, only 2 isolations have been known, namely *Rhizoctonia* sp. and *Tulasnella* sp., while the rest were not identified yet. Among them, the DNA of the 11 isolations were able to be extracted for further analysis. The constructed phylogenetic tree performed that those species could be grouped into 4 major clusters. Two species, *Rhizoctonia* sp. and *Tulasnella* sp. were in different clusters.

Key words: *Spathoglottis plicata*, orchid-mycorrhiza, *Rhizoctonia*, *Tulasnella*, Papua

INTRODUCTION

Spathoglottis plicata Blume (ground orchid) is one of the orchid species that is widely distributed in the world, including in the area of New Guinea (Papua New Guinea, PNG and Papua, Indonesia). According to Agustini et al. (2008, 2009) Cycloop Mountains Nature Reserve (CAPC) is one of the conservation area in Papua. This species of orchid is also found here. One of buffer zone areas of this nature reserve is a University of Cenderawasih (Uncen) campus forest in Waena, Jayapura. It has plenty of *S. plicata* orchids. This orchid is easy to grow and has purple-white flowers and bloom throughout the year.

In nature, for the germination and early growth of orchid seed and its development, it needs an association of orchid roots and mycorrhizal fungi (orchid-mycorrhiza) (Warcup 1981; Cameron et al. 2006; Suarez et al. 2006; Agustini et al. 2009). This is because orchid seeds are very small, smooth and soft and have no cotyledon which is a food reserve in the early growth of seeds (Sarwono 2002; Agustini and Kirenius 2002). The association to mycorrhizal fungi helps the orchid in providing the nutrients needed for the process of germination of its seeds (Agustini and Kirenius 2002; Agustini 2003). The hyphae of mycorrhizal fungi penetrate to the cell walls of roots and then will be developed in the root cortex as dense coiled called peloton. Mycorrhizal hyphae are very delicate; therefore they will absorb water and certain minerals (Suarez et al. 2006; Agustini et al. 2009).

The role of mycorrhizal fungi is very interesting in the life cycle of orchids. In the early growth, all orchid species are heterotrophic, so it requires association with mycorrhizal fungi to obtain the needed nutrients (Brundrett et al. 2003; Taylor et al. 2004; Wu et al. 2010). Orchids that have low levels of heterotrophic are dependants on the presence of mycorrhizae. Based on morphological characteristics, most of the fungi associated with orchids are members of the form of anamorphic (asexual)-genus *Rhizoctonia* (Athipunyakom et al. 2004) and generally come from families of Basidiomycetes. Several studies of terrestrial orchid-mycorrhiza in temperate-climate areas have been done a lot, but the studies on the tropics are very few (Otero et al. 2002). In Papua, researches and publications on ground orchid-mycorrhiza, especially *S. plicata* are very small in number. Therefore, there is a need for the study on the diversity of orchid-mycorrhiza associated with ground orchids *S. plicata*. Furthermore, according to Kristiansen et al. (2001) to find out the phylogenetic of the obtained isolates, the DNA sequencing analysis is required. Therefore, the purpose of this study was to isolate and to find out the phylogenetic relationship of the orchid-mycorrhiza of *S. plicata* species using mitochondrial sequences of ribosomal large subunit (mt-Ls) DNA.

MATERIALS AND METHODS

Time and area study

The research was carried out for 10 months in 2008. The isolation of orchid-mycorrhiza was done in the laboratory of Plant Tissue Culture and Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Cenderawasih (Uncen), Jayapura, Papua, Indonesia. The maintenance and regeneration of the isolates were carried out at the same place.

Isolation of orchid-mycorrhiza

According to Manoch and Lohsomboon (1992), isolation of orchid-mycorrhiza was done by the modified method of Masuhara and Katsuya (1989). The roots of *S. plicata* was cut for about 1 cm, and sterilized with 10% of Clorox for 30 seconds, and then washed with aquadest and rinsed with distilled water, then sterilized again with 70% alcohol. The sterilized pieces of roots were sliced transversely using a sterile knife (razor blade) in the LAF (Laminar Air Flow). The thickness of the slice was ± 200-300 µm or 3-4 pieces in 1 mm slice. The slices then were planted in the media of *potato dextrose agar* (PDA) in petri dish. Each dish were filled with 1-3 pieces of roots and then incubated at 28^{o}C. Mycorrhizal mycelia start to appear within 2-3 days.

After mycorrhizae grew, isolation was conducted if, through a morphology observation, there was a difference in shapes and growth patterns, to separate each of these groups, because there possibly was more than one species of media. In the same medium, the isolation was carried out again if in the growing up process, the different shape and growth patterns can still be found, until finally a really pure and free from contamination-free isolate was obtained (each mycorrhizal was cultured separately). In 2-3 times of isolation, the isolates obtained were really pure.

Identification of orchid-mycorrhiza

The identification of the species of orchid-mycorrhiza was done by looking at the morphological and microscopic characteristics. Morphological features, including the diameter of colony growth, mycelia morphology and other characteristics. Microscopic characteristics include presence or absence of septum and spores shape or conidia. Some of the literatures used in the identification of orchid-mycorrhiza were Athipunyakom et al. (2004) and Karl-Franzens (2006).

Isolates of orchid-mycorrhiza and phylogenetic relationship

The isolates culture and DNA extraction

The mycelia of each isolate was dissolved in 2 µL of aquadest and transferred into 0.5 mL Eppendorf tubes containing 6 µL of dd H2O and 2 µL of 10X PCR (polymerase chain reaction) buffer (0.35 M Sorbitol, 0.1 M Tris, 5 mM EDTA pH 7.5). The solution was then heated at 95 ^{o}C for 10 minutes in a water bath. The DNA of these isolates was extracted using the method of hexadecyl-trimethylammonium bromide (CTAB) (Gardes and Bruns 1993). The DNA quality was checked by electrophoresis.

DNA amplification by PCR

Of the extracted Isolates, 8 µL was used as a template for PCR amplification. ML5/ML6 regions of the genes of mitochondrial large submit (mt-Lr) RNA was amplified with a PCT100 thermocycler (MJ Research Inc.., MA). PCR solution which was used for each isolates contains 2 µL of 10X PCR Buffer, 2 µL (mM) for each primer, 6 µL ddH_2O, 8 µL dNTPs (2 mM) and 0.1 µL Taq polymerase. PCR was conditioned according to the method of Gardes and Bruns (1993): initial denaturation at temperature of 95^{o}C for 1 minute 35 second, followed by 13 cycles of denaturation for 35 seconds, temperature 94 ^{o}C, primer annealing for 55 seconds at a temperature of 55 ^{o}C, and polymerization for 45 seconds at 72. The addition of nine cycles of the polymerization was done by extending to 2 minutes. The 9 cycles consisting of 3 min of extension, and ending with 10 min of polymerization at a temperature of 72^{o}C. Primer used was ML5 (CTCGGCAAATTATCCTCATAAG), MLIN3 (CGACACAGGTTCGTAGGTAG) and MI6 (CAGTAGAAGCTGCATAGGGTC).

DNA sequencing

The results of PCR amplification was purified with QIA Quick PCR extraction kit (Qiagen GmbH GE). DNA was dissolved in 30 µL of sterile H_2O and was being sequenced with a primer ML5/ML6 using ABI 377 automatic sequencer (Perkin Elmer) (Kristiansen et al. 2001). The sequencing uses the dideoxy chain termination procedure (Sanger et al. 1977).

RESULTS AND DISCUSSION

Isolation of orchid-mycorrhiza

The results of orchid-mycorrhiza isolation associated with *S. plicata* on eight sites are 13 isolates. Of these 13, 2 of them morphologically are identified as *Rhizoctonia* sp. and *Tulasnella* sp., while 11 other species of orchid-mycorrhiza, namely sp.1, sp.2, sp.3, sp.6, sp.7, sp.8, sp.9, sp.10, sp.11, sp.12, and sp.13 isolates are associated with *S. plicata* which is only from 1 different location (Table 1) not all of them can be identified based on differences in morphological features (Figure 1). However, the results of phylogenetic analysis (Table 2, Figure 2) shows that there are 4 clusters that are likely encountered two other species of orchid-mycorrhiza.

The results of this isolation adds the data of isolation result of the initial survey conducted by Agustini et al. (2009) in the campus of Faculty of Mathematics and Natural Sciences, Cenderawasih University, located in the buffer zone of CAPC. In her research, she discovered two species of orchid-mycorrhiza, namely *Rhizoctonia* sp. and *Tulasnella* sp which are isolated from orchids *S. plicata* and several other species of orchids. In this research, at least there are two unknown species of orchid-mycorrhiza. Both species are different from *Rhizoctonia* sp. and *Tulasnella* sp.

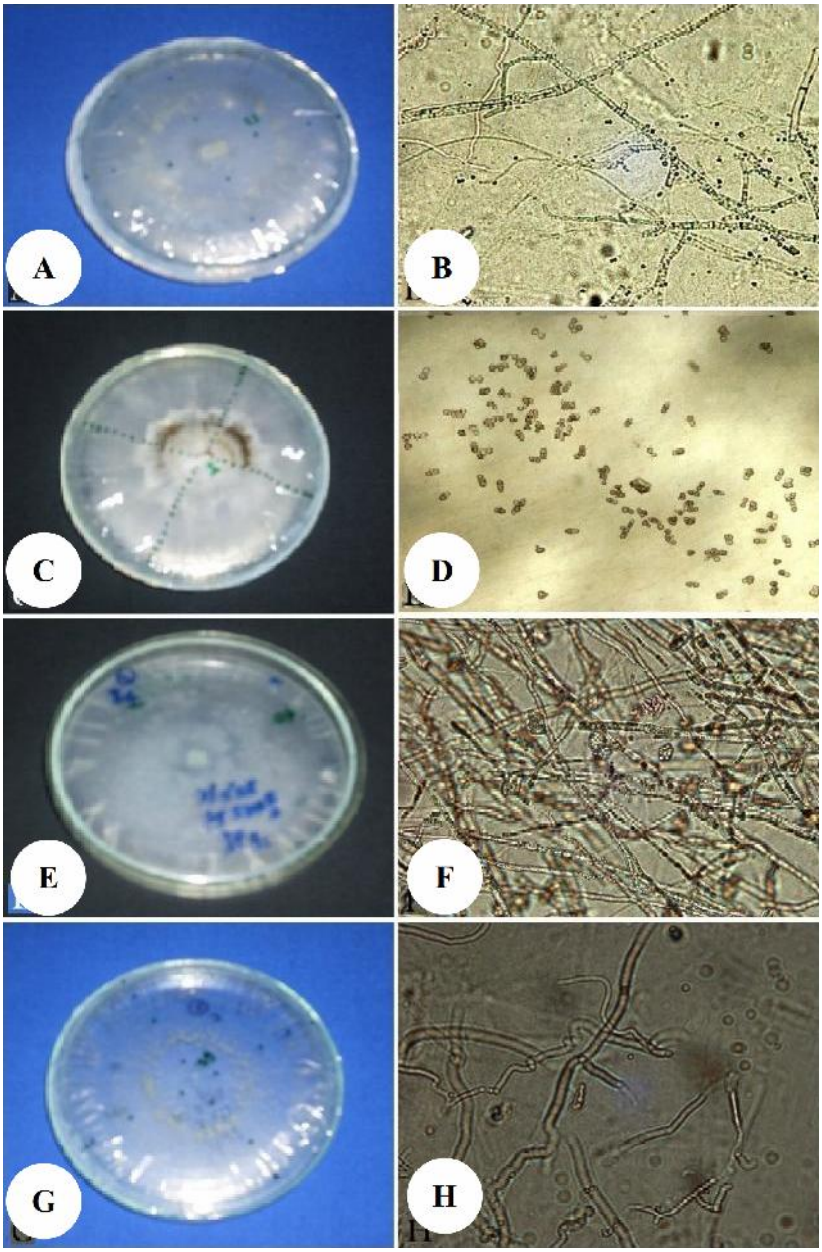

Figure 1. Morphology of some isolates of orchid mycorrhiza isolated from *S. plicata*. A-B: Isolates sp7., C-D: Isolate sp.2., E-F: *Tulasnella* sp., and G-H: *Rhizoctonia* sp.

Table 1. Species of orchid-mycorrhiza from *S. plicata* in the Campus area Cenderawasih University, Waena, Jayapura.

Isolates of orchid-mycorrhiza	Code of sampling location							
	L1	L2	L3	L4	L5	L6	L7	L8
Rhizoctonia sp.	√		√	√			√	√
Tulasnella sp.	√	√			√			
sp.1	√							
sp.2	√							
sp.3	√							
sp.6		√						
sp.7						√		
sp.8						√		
sp.9					√			
sp.10						√		
sp.11					√			
sp.12						√		
sp.13							√	
	5	2	1	1	3	4	2	1

Note: √ = found; - = not found; L1 to L8 = sites in Waena, Jayapura

In a study, Athipunyakom et al. (2004) isolated orchid-mycorrhiza of *S. plicata* obtained from Chiang Mai, Chanthaburi, Nakhon Ratchasima and Bangkok and found three genera and four species of orchid-mycorrhiza which has successfully been identified based on morphological characteristics, namely *Epulorhiza repens*, *Rhizoctonia globularis*, *Rhizoctonia* sp., and *Sebacina* sp.

Table 1 show that *S. plicata* can be associated with more than one species of mycorrhiza-orchid. Kristiansen et al. (2001) showed that the orchid can be associated with more than one species of mycorrhizal fungi. In addition, Handayanto and Hairiah (2007) also said that some mycorrhizal fungi can colonize one root of the plant. This is because identification of orchid- mycorrhiza fungi in conventional way is still difficult to conduct. According to Rasmussen (2004), Hollick et al. (2004) and Kristiansen et al. (2001) the characteristic of the fungus which never reach sexual phase or sterile in a culture condition made it difficult for identification process so that the identification with molecular techniques needs to be done.

Description of orchid-mycorrhiza species

Orchid-mycorrhiza orchids isolated from *S. plicata*, showing the development of a variety. The most rapid growth of the colony diameter reached by isolates sp.2 with a diameter of 9 cm in 3 days, followed by *Rhizoctonia* sp., *Tulasnella* sp., sp.12 and sp.1. While other species of orchid- mycorrhiza with colony growth of more than 15 days showed a very slow growth, whereas the optimum diameter of colony growth for orchid-mycorrhiza is 15 days. The growth rapidity of this colony affects the speed of nutrients absorption in nature. According to Smith and Read (2008), the growth rapidity of mycelia/hyphae potentially increased the extent of nutrients absorption surface in the soil during symbiosis process with the plant root system. Thus, the plants quickly obtained nutrients needed through hyphae.

Table 2. Description of the morphology of orchid-mycorrhiza species associated with *S. plicata*.

Mycorrhiza (isolates) species	Growth on PDA	Morphology
Rhizoctonia sp.	▪ The growth of the colony very fast, within 4 days. ▪ Mycelia are translucent white and thin. There is a set of dark green granules forming a round shape in the middle.	▪ Branched hyphae, less dense ▪ At the end of hyphae branch, it is formed small sphere-shaped granules, which in turn form a collection of granules. The presence of globulus oil is found around it.
Tulasnella sp.	▪ Growth of colonies is rather slow, 8 days. ▪ Mycelia are white and thicker.	▪ Branched hyphae with dense septate. ▪ The presence of conidia, in a group or scattered, and with a magnification of 1000x, the conidia seems like bananas, in sectional.
sp.1	▪ Colony growth is slow. ▪ Achieving a diameter of 9 cm in 12 days. ▪ Mycelia are whitish-green brown. ▪ Mycelia are thick and round.	▪ Branched hyphae, more than 1core, dense septate. ▪ Spores are brown and on the tip of hyphae. ▪ The hyphae are long and round and in the shape of chain, and the conidia are round, semi round, ramiform (like kidney shapes), allantoidal (like cashew nut shape with no-sharp edge), and crystal look-like.
sp.2	▪ The colony growth is very fast, within 3 days ▪ Blurred white mycelia	▪ Mycelia are rather thick and the granules are yellow to green and are scattered in a circle in the middle and edge. ▪ Microscopically, hyphae are branched with long septet. There are dispersed small oval-shaped spores.
sp.3	▪ The colony growth is very fast, within 5 days ▪ Mycelia are white to yellow and have tree-like branches when mature.	▪ The hyphae are septate and dense. At the tip of the hyphae, there are round and brown spores, and also in "V" like shape in brown color with parallel lines in it.
sp.6	▪ Colony growth is very slow, reaching only Ø 9 cm in 22 days ▪ Mycelia are yellowish green, thick and form concentric zones. On the edge side, mycelia are thin and white.	▪ Branched hyphae with dense septate. ▪ Spores are clustered and dispersed small round shape. Some tips of hyphae form many branches covered by the collection of spores.
sp.7	▪ Slow colony growth, 15 days ▪ Mycelia are white with dark brown sides that form a circle in the middle and thick.	▪ The hyphae are branched with non-close septate. ▪ Conidia's shape are like crystals and resembles a figure of number eight are scattered and there is a chain composed ellipse.
sp.8	▪ Colony growth is slow, 17 days ▪ Mycelia are white with brown color in the middle, and appear thicker	▪ Hyphae are long with septate. ▪ Conidia are round and crystals-shaped with elongated dots which are arranged like a chain. ▪ The presence of large round-shaped spores is found on the tip of hyphae.
sp.9	▪ Colony growth is very slow 20 days ▪ At the beginning, Mycelia grow clearly white and thin. As the growth continues, white mycelia appear thicker in the middle.	▪ branched hyphae with non-close septate. With 400x magnification, the hyphae look like spiral. ▪ Some hyphae appear thickened and the presence of spiny conidia is visible and scattered round.
sp.10	▪ Growth of colony is very slow, 24 days. ▪ Mycelia are white with blackish brown in the middle and it looks thicker.	▪ There are branched hyphae and close septate. ▪ The shapes of conidia are like crystals, and there is much globular oil which is attached to the hyphae.
sp.11	▪ Slow growth colony, 16 days. ▪ Mycelia are thick and white forming concentric zone.	▪ Hyphae are septate and branched. ▪ Some hyphae appear thickened and there are many globular attached to the hyphae. Among hyphae, there are conidia in crystal shape.
sp.12	▪ Colony growth is rather slow, 8 days ▪ Mycelia are blackish green and thick.	▪ Hyphae are branched with non-close septate. ▪ Many spores are black round-shaped and are found on the tip of hyphae. Some hyphae are coil-shaped.
sp.13	▪ Growth of colonies is very slow; it needs more than a month to reach a diameter of 9 cm. ▪ Mycelia are white and thick.	▪ Hyphae have long septate and a lot of branches. ▪ There are many scattered elliptical conidia and are attached to the hyphae. ▪ In addition, there is presence of globular attached to the hyphae

Table 3. Phylogenetic relationship of 11 isolates of orchid orchid-mycorrhiza based on molecular approach (DNA) isolated from orchid *S. plicata*.

Isolate	1	2	3	4	5	6	7	8	9	10	11
1	****	0.6667	0.3333	0.3333	0.3333	0.6667	1.0000	0.6667	0.6667	0.3333	0.3333
2	0.4055	****	0.6667	0.6667	0.6667	1.0000	0.6667	1.0000	1.0000	0.6667	0.6667
3	1.0986	0.4055	****	0.3333	1.0000	0.6667	0.3333	0.6667	0.6667	0.3333	1.0000
4	1.0986	0.4055	1.0986	****	0.3333	0.6667	0.3333	0.6667	0.6667	1.0000	0.3333
5	1.0986	0.4055	0.0000	1.0986	****	0.6667	0.3333	0.6667	0.6667	0.3333	1.0000
6	0.4055	0.0000	0.4055	0.4055	0.4055	****	0.6667	1.0000	1.0000	0.6667	0.6667
7	0.0000	0.4055	1.0986	1.0986	1.0986	0.4055	****	0.6667	0.6667	0.3333	0.3333
8	0.4044	0.0000	0.4055	0.4055	0.4055	0.0000	0.4055	****	1.0000	0.6667	0.6667
9	0.4055	0.0000	0.4055	0.4055	0.4055	0.0000	0.4055	0.0000	****	0.6667	0.6667
10	1.0986	0.4055	1.0986	0.0000	1.0986	0.4055	1.0986	0.4055	0.4055	****	0.3333
11	1.0986	0.4055	0.0000	1.0986	0.0000	0.4055	1.0986	0.4055	0.4055	1.0986	****

Phylogenetic relationship of orchid-mycorrhiza of *Spathoglottis plicata*

In Table 2, the isolates number of mycorrhizal orchid which were isolated from *Spathoglottis* plants are 13 species, but only 11 species that can be isolated their DNA. Therefore, phylogenetic analysis (Table 3) would only be done on these 11 species that have been successfully analyzed. The results of DNA analysis shows that there are four major groups (clusters) of 11 species of orchid-mycorrhiza isolated from *S. plicata* (Figure 2). With the method of UPGMA (unweighted pair group method with arithmetic mean), cluster 1 shows the phylogenetic ties among sp.1 and sp.7 isolates. Cluster 2 shows the relationship among the four species, namely, sp.2, sp.6, sp.8 and sp.9 isolates. Cluster 3 shows that there are 3 isolates having a very close relationship, namely sp.3 isolates, *Tulasnella* (*Tul*) and sp.11 isolates. Whereas cluster 4 shows that there are two species of isolates having a close relationship ties i.e. the species of *Rhizoctonia* (*Rhi*) and sp.10 isolates. And also, the results show that each isolates of those four clusters have the same index value of relationship. *Rhizoctonia* has a relationship index equal to 100% to isolates sp.10, *Tulasnella* to isolates sp.3 and sp.11, so it can be said that these isolates have the same species.

The growth rate of the colonies did not affect the nearby distant phylogenetic relationship in one cluster. It can be seen from the *Tulasnella*, which grows slowly (8 days/9 cm), is different from sp.11, which has a diameter of colony growth on PDA medium for up to 16 days/9 cm, although morphologically the relationship among species and isolates within a cluster is very close. It is similar with *Rhizoctonia* case, morphologically, it grows very fast (4 days) on PDA medium, whereas sp.10 grows very slow and it takes about 24 days to reach a diameter of 9 cm. However, microscopically both are similar in its morphology (Table 1). The same thing occurs in clusters 1 and 2. For the species group of the two clusters, the DNA relationship is closer to cluster 3 of *Tulasnella* sp. and is further to *Rhizoctonia* sp. group.

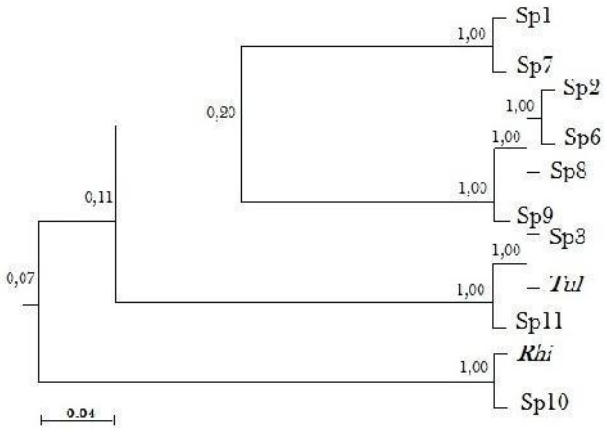

Figure 2. Dendrogram showing the phylogenetic relationship of several species and isolates of orchid-mycorrhiza isolated from *S. plicata* with UPGMA method.

CONCLUSION

Based on morphological and microscopic character, there were 13 isolates of orchid mycorrhizal fungi has been isolated from *S. plicata*'s root. Among them, isolates has been known as *Rhizoctonia* sp and sp *Tulasnella*. The constructed phylogenetic tree of 11 isolates performed that those species could be grouped into 4 major clusters. Two species, *Rhizoctonia* sp. and *Tulasnella* sp. were in different clusters.

ACKNOWLEDGEMENTS

The authors gratefully thank Maria Imco, Ira Ardila Putri, and Dewi Katemba, which has helped the research at the Laboratory of Biological Science University of Cenderawasih, Papua; to the General Director of Higher Education that has funded this research through Fundamentals Grant of 2008. To Prof. Zamir K. Punja

(Simon Fraser University, Burnaby, Canada), thanks for the help during the discussion of this matter.

REFERENCES

Agustini V, Kirenius M. 2002. The effect of mycorrhiza on early growth and development of *Dendrobium* sp. Sains 2 (2): 55-60. [Indonesia]

Agustini V, Sufaati S, Suharno. 2008. Terrestrial orchid in Mt. Cycloops Nature Reserve, Jayapura-Papua. The 9th New Guinea Biology Conference. Jayapura, 24-26 July 2008.

Agustini V, Sufaati S, Suharno. 2009. Mycorrhizal association of terrestrial orchids of Cycloops Nature Reserve, Jayapura. Biodiversitas 10 (4): 175-180.

Agustini V. 2003. The role of orchid-mycorrhiza on growth of *Dendrobium* sp. Sains 3 (2): 39-42. [Indonesia]

Athipunyakom P, Manoch L, Piluek C. 2004. Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Nat Sci 38 (2): 216-228.

Brundrett MC, Scade A, Batty AL, Dixon KW, Sivasithamparam K. 2003. Development of in situ and ex situ seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. Mycol Res 107 (10): 1210-1220.

Cameron DD, Leake JR, Read DJ. 2006. Mutualistic mycorrhiza in orchids. Evidence from plant-fungus carbon nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol 171: 405-416.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes: Application to the identify cation of mycorrhizae and rusts. Mol Ecol 2: 113-118.

Handayanto E, Hairiyah K. 2007. Soil Biology: Healthy Soil Management Platform. Pustaka Adipura, Yogyakarta. [Indonesia]

Hollick PS, Taylor RJ, McComb JA, Dixon KW, Krauss SL. 2004. Optimisation of DNA extraction for AFLP analysis of mycorrhizal fungi of terrestrial orchids Caladeniimae and Drakaeinae. Pl Mol Biol Rep 22: 307a-307h.

Karl-Franzens. 2006. Mycorrhizal fungi of terrestrial orchids. Institute of Botany, University Graz, Germany.

Kristiansen KA, Taylor DL, Kjoller R, Ramussens HN, Rosendahl S. 2001. Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-stand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Mol Ecol 10: 2089-2093.

Manoch L, Lohsomboon P. 1992. Isolation of mycorrhizal fungi from orchid roots. Chiang Mai University Regional Training Course on Biology and Technology of Mycorrhiza 2: 5, SEAMEO BIOTROP, Bogor.

Masuhara G, Katsuya K. 1989. Effects of mycorrhizal fungi on seed germination and early growth of three Japanese terrestrial orchids. Scientia Horticulturae 37: 331-337.

Otero JT, Ackerman JD, Bayman P. 2002. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. Am J Bot 89 (11): 1852-1858.

Rasmussen HN. 2004. Recent developments in study of orchid mycorrhiza. Plant and Soil 244 (2): 149-163.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Biotechnology 24: 104-8.

Sarwono B. 2002. Identify and making hybrid orchids. PT Agro Media Pustaka. Jakarta. [Indonesia]

Smith SE, Read D. 2008. Mycorrhizal symbiosis. 3rd ed. Academic Press, New York.

Suarez JP, Weiβ M, Abele A, Garnica S, Oberwinkler F, Kottke I. 2006. Diverse tulasnelloid fungi from mycorrhizas with epiphytic orchids in an Andean cloud forest. Mycol Res 110: 1257-1270.

Taylor DL, Bruns TD, Hodges SA. 2004. Evidence for mycorrhizal races in a cheating orchid. Proc Biol Sci 271 (1534): 35-43.

Warcup JH. 1981. The mycorrhizal relationship of Australian orchids. New Phytol 87 (2): 371-381.

Wu J, Ma H, Lu M, Han S, Zhu Y, Jin H, Liang J, Liu L, Xu J. 2010. *Rhizoctonia* fungi enhance the growth of the endangered orchid *Cymbidium goeringii*. Botany 88: 20-29.