http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2016.299.305

Research Article
Growth Effect of *Cinnamomum kanehirae* Cuttings Associated with its Dark Septate Endophytes

Lei-Chen Lin

Department of Forestry and Natural Resources, National Chiayi University, Chiayi, 60004 Taiwan, Republic of China

Abstract

Background and Objective: Stout camphor tree (*Cinnamomum kanehirae* Hay.) is an endemic specie in Taiwan and cutting is the major propagation of *C. kanehirae* for plantation. Mycorrhiza can accelerate the growth of the host plant, especially in root of the host plant. The objective of this study was to investigate the growth effect of the 2 dark septate endophytes isolated from *C. kanehirae*. **Materials and Methods:** To measure the effects of stains CkDB2 and CkDB5 on growth performance of cuttings, the cuttings were carefully removed from their substrate after 9 months of incubation. Each treatment had three replicates. **Results:** After 9 month incubation, the mycorrhizal synthesis experiment showed that the roots of synthesized cuttings produced microsclerotia, a characteristic of dark septate endophyte, but nothing was found in the control. All inoculated cuttings had higher values of net height growth, dry weight, leaf area and chlorophyll concentration than the control. **Conclusion:** This study demonstrated that the 2 endophytes, strains CkDB2 and CkDB5, capable of forming microsclerotia with *C. kanehirae* cuttings were dark septate endophytes. Based on the results, CkDB5 had a better growth response than CkDB2. Cuttings inoculated with CkDB5 showed a 200% increase in the root dry weight and therefore, CkDB5 could presumably be a prerequisite for the survival of *C. kanehirae* cutting plantation.

Key words: *Cinnamomum kanehirae*, cutting, dark septate endophyte, growth response, microsclerotia

Received: May 09, 2016 Accepted: May 26, 2016 Published: June 15, 2016

Citation: Lei-Chen Lin, 2016. Growth effect of *Cinnamomum kanehirae* cuttings associated with its dark septate endophytes. Pak. J. Biol. Sci., 19: 299-305.

Corresponding Author: Lei-Chen Lin, Department of Forestry and Natural Resources, National Chiayi University, Chiayi, 60004 Taiwan, Republic of China

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cinnamomum kanehirae Hay., a member of Lauraceae is an endemic specie and one of the 5 precious hardwood trees in Taiwan[1]. *Antrodia camphorata* is a well-known medicinal polypore found only in *C. kanehirae*[2]. Thus, *C. kanehirae* has been widely felled and is now an endangered species[3,4].

Cinnamomum kanehirae seeds have an entomophilous characteristic and cannot be easily collected, hence, cutting is the best silvicultural practice for the resource conservation of *C. kanehirae*[2,3]. Kao and Huang[5] indicated that cutting *C. kanehirae* could promote the formation of adventitious roots and got the best rooting rate of up 80%. However, using cutting for forestations often fails. The major factor for such failure is the lack of axial roots[6]. Hence, increasing the survival rate of *C. kanehirae* plantation is a key point for its conservation.

The various forms of mycorrhizal associations are ubiquitous in nature and play important roles in plant nutrition and nutrient cycling. They also influence the structure and dynamics of the plant communities within which they exist[7]. Functions of mycorrizae are to promote growth[8,9] and tolerance[10,11] of the host plant. Mycorrizae can increase rooting, with increased plant rooting, the growth of the host plant is promoted, especially in cutting[12-17]. Scagel[14,16] also demonstrated that cuttings inoculated with mycorrhizal fungi had greater root initiation than non-inoculated cuttings.

Although, there are many studies on the indigenous *C. kanehirae* of Taiwan, most focused on the physiological ecology, phylogenetic relationships[18-20] and propagation of the genus[3,5,21,22], little attention has been paid to *C. kanehirae* mycorrhizae[4,20,23,24]. Two kinds of mycorrhiza, Arbuscular Mycorrhizae (AM) and Dark Septate Endophyte (DSE) have recently been discovered in *C. kanehirae*[20]. The AM fungi are obligate biotrophs and cannot be cultured without plants[25], but dark septate endophytes can be grown in solid or liquid media[20]. Lin *et al.*[4], demonstrated that *C. kanehirae* cuttings could resynthesize with its root fungus endophytes to form the structure of dark septate endophyte. However, the benefits of these 2 endophytes associated with *C. kanehirae* cuttings remain unknown. The objective of this study was to investigate the growth efficiency and physiological characteristics of *C. kanehirae* and its dark septate endophytes.

MATERIALS AND METHODS

Strains: Two dark septate endophytes were isolated previously from the roots of *C. kanehirae* (120°47'37.33" E, 23°28'16.12" N). The plantation was located in Dabang Township, Chiayi County, Taiwan[20]. Specimens of CkDB2 and CkDB5 were deposited at the Tree Mycorrhiza Laboratory of National Chiayi University and their Internal Transcribed Spacer (ITS) genomic sequences were deposited in the GenBank (CkDB2 and CkDB5 isolates, KT780305 and KT780306, respectively)[4].

Cuttings: Two year-old cuttings of *C. kanehirae* were obtained from Jhongsing Nursery (Chiayi Forest District Office, Forest Bureau, Council of Agriculture, Executive Yuan, Chiayi, Taiwan) and transplanted in new pots (10 cm diameter) in a nursery substrate (peat and perlite 3:1 v/v, previously autoclave-sterilized at 121°C for 60 min).

Inoculation with endophytes: The inoculation was performed according to the method of Zhang *et al.*[26] with modifications. In brief, the cuttings were transplanted into new pots with the inoculum (10 pieces) placed near the root. The three treatments (1 control and 2 inoculated treatments) each had 3 replicates. The cuttings were grown, watered and fertilized in a greenhouse. Standard fertility was given to each pot with 50 mL sterile Hoagland's nutrient solution every week.

Staining of root: After 9 months of incubation, the cuttings were removed from the pots. The roots were washed thoroughly with tap water, cut into segments of approximately 1 cm long, cleaned in 10% (w/v) KOH and 3% H_2O_2 according to the degree of lignification of the roots and stained with trypan blue lactophenol. The method was modified according to that used by McGonigle *et al.*[27]. Observation was made under light microscopy (Olympus X5).

Plant growth responses: To measure the effects of stains CkDB2 and CkDB5 on growth performance of cuttings, the cuttings were carefully removed from their substrate after 9 months of incubation. Each treatment had 3 replicates.

The net growth of height and root collar diameter of all treatments were regularly recorded after the cuttings were transplanted. The last measurement was taken right before the harvest.

The plant dry weight (root, stem and leaf) was obtained by oven-drying at 80°C until constant weight. Leaf Area (LA) of fresh leaves was determined using a leaf area meter (LI-COR LI 3100). Leaf Area Ratio (LAR), Specific Leaf Area (SLA) and Leaf Weight Ratio (LWR) were calculated using leaf area and dry weights of harvested plants. The equations used are as follows:

$$LAR = \frac{\text{Leaf area } (cm^2)}{\text{Total dry weight (g)}}$$

$$SLA = \frac{\text{Leaf area } (cm^2)}{\text{Leaf dry weight (g)}}$$

$$LWR = \frac{\text{Leaf dry weight (g)}}{\text{Total dry weight (g)}}$$

Plenchette *et al.*[28] defined the Mycorrhizal Dependency (MD) as the degree to which a plant is dependent on the mycorrhizal condition, which is expressed as follows:

$$MD = \frac{\text{(Dry weight of mycorrhizal plant-dry weight of non-mycorrhizal plant)}}{\text{(Dry weight of mycorrhizal plant)}} \times 100$$

Chlorophyll concentration: The method was modified according to that used by Porra[29]. To measure chlorophyll concentration, the third and 4th fully-expanded leaves (counting from the apex) were collected (two leaves per plant, n = 3). Then, 0.05 g of each sample (fresh material) was grounded with 10 mL of 80% acetone. The suspension was filtered and measured by a spectrophotometer (spectrophotometer, Hitachi U-2000) in the wavelengths of 663 nm (Chlorophyll a: Chl a) and 645 nm (Chlorophyll b: Chl b).

Chl a = (12.7×D663-2.69×D645)×(V/1000W)
Chl b = (22.9×D645-4.68×D663)×(V/1000W)
Chl a+b = (20.2×D645+8.02×D663)×(V/1000W)
Dλ = Stands for absorbency at the specific wavelength λ
V = Volume of ground leaf-acetone liquid (mL)
W = Volume of the fresh weight (g)

Statistical analysis: Statistical analysis was performed using the software Statistical Package for the Social Sciences (SPSS 12.0) (Illinois, USA) for windows program. Means of 3 separate experiments±standard error (n = 3) were derived from all collected data. Differences among endophytes were analyzed by Turkey's multiple range test at $p \leq 0.05$ significant level.

RESULTS AND DISCUSSION

Morphology and colonization: After 9 months of incubation, all cuttings survived. However, the inoculated cuttings showed better growth than the control (Fig. 1).

In the observation of stained root, microsclerotia (Fig. 2a), a feature of dark septate endophyte were found in the root associations of CkDB2-inoculation. Microsclerotia were also found in the CkDB5-inoculation (Fig. 2b), but noting was founded in the control (Fig. 2c). Therefore, the 2 endophytes could be associated with *C. kanehirae* cuttings and the root associations had the feature of dark septate endophyte. Similar results were also obtained in other studies[30-33].

Plant growth responses

Net growth of height and root collar diameter: Both net growth of height and root collar diameter were measured after 9 months of incubation (Table 1). The cuttings inoculated with strains CkDB2 and CkDB5 showed the greatest net height growth (10.33±0.95 and 11.73±1.04 cm, respectively), compared with the control (2.67±0.21 cm). The net height growth of all treatments were significantly higher than that of the control ($p<0.05$), but the average net growth of root collar diameter of all treatments showed no significant difference.

Plant dry mass: At harvest, there was significant difference in dry weight (root, leaf and total dry weight) of all treatments (Table 2). The CkDB2-inoculation and CkDB5-inoculation generated better root dry weight values (11.30±1.48 and 14.44±0.72 g, respectively) than the control (6.33±2.25 g), revealing statistically significant difference ($p<0.05$). The leaf dry weight and the total dry weight showed the similar results. The CkDB2-inoculation and CkDB5-inoculation produced better leaf dry weight values (6.44±1.34 and 7.32±0.65 g, respectively) than the control (2.72±0.31 g). The CkDB2-inoculation and CkDB5-inoculation also yielded better total dry weight values (26.07±3.92 and

Table 1: Growth effect of *Cinnamomum kanehirae* cuttings with different treatments after 9 month incubation

	Net growth (cm)	
Treatments	Height	Root collar diameter
Ck	2.67±0.21[a]	0.21±0.12[a]
CkDB2	10.33±0.95[b]	0.33±0.24[a]
CkDB5	11.73±1.04[b]	0.36±0.03[a]

All values were Means±Standard Deviation of three replicate cultures, values in the same column with different letters were different at 5% significant level

Table 2: Dry weight of *Cinnamomum kanehirae* cuttings with different treatments after 9 month incubation

	Dry weight (g)			
Treatments	Root	Stem	Leaf	Total
Ck	6.33±2.25[a]	4.90±1.90[a]	2.72±0.31[a]	13.94±3.58[a]
CkDB2	11.30±1.48[b]	8.34±1.35[a]	6.44±1.34[b]	26.07±3.92[b]
CkDB5	14.44±0.72[b]	8.47±1.00[a]	7.32±0.65[b]	30.23±2.16[b]

All values were Means±Standard Deviation of three replicate cultures, values in the same column with different letters were different at 5% significant level

Fig. 1(a-c): Morphology of mycorrhizal synthesis of *Cinnamomum kanehirae* cuttings after 9 month incubation, (a) CkDB2-inoculation, (b) CkDB5-inoculation and (c) Control

Table 3: Leaf area and leaf parameters of *Cinnamomum kanehirae* cuttings with different treatments after 9 month incubation

		Leaf parameters		
Treatments	Leaf area (cm^2)	LAR	SLA	LWR
Ck	279.4±31.6^a	20.64±3.52^a	102.8±1.7^a	0.20±0.03^a
CkDB2	602.9±23.1^b	23.48±3.57^a	96.6±21.1^a	0.25±0.02^a
CkDB5	845.5±74.3^c	25.39±7.78^a	115.7±8.0^a	0.22±0.09^a

All values were Means±Standard Deviation of three replicate cultures, values in the same column with different letters were different at 5% significant level, LAR: Leaf area ratio, SLA: Specific leaf area and LWR: Leaf weight ratio

30.23±2.16 g, respectively) than the control (13.94±3.58 g). However, there were no significant differences in stem dry weight of the control, CkDB2 and CkDB5-inoculatin (4.90±1.90, 8.34±1.35 and 8.47±1.00 g, respectively).

Leaf area, leaf area ratio, specific leaf area and leaf weight ratio: The leaf area of cuttings was significantly higher when stimulated by inoculation (Table 3) ($p<0.05$). The CkDB2 and CkDB5-inoculation produced greater leaf area (602.9±23.1 and 845.5±74.3 cm^2, respectively) than the control (279.4±31.6 cm^2), but there were no significant differences in LAR, SLA and LWR.

Chlorophyll concentration: Chlorophyll concentration of all treatments were analyzed at harvest (Table 4). Both inoculations had significantly difference compared with the control ($p<0.05$). For Chl a concentration, CkDB2 and CkDB5-inoculation (0.50±0.17 and 0.54±0.04 mg g^{-1},

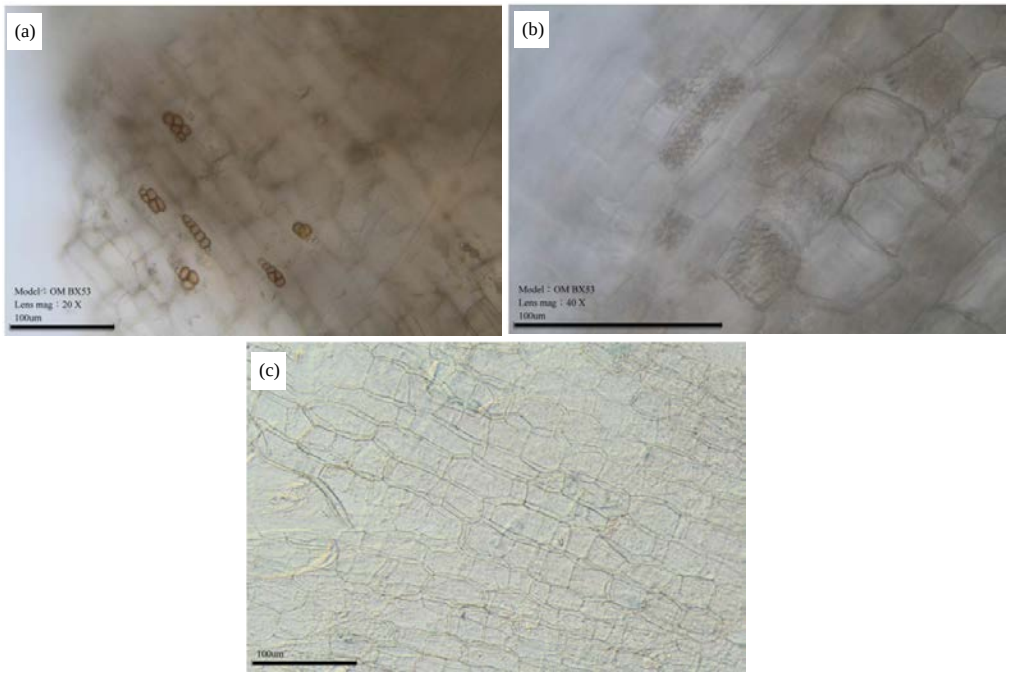

Fig. 2(a-c): Root structure of *Cinnamomum kanehirae* cuttings after 9 month incubation, (a) CkDB2-inoculation, (b) CkDB5-inoculation and (c) Control

Table 4: Chlorophyll concentration in leaves of cuttings with different treatments after 9 months incubation

	Chlorophyll concentration (mg g^{-1})		
Treatments	Chl a	Chl b	Chl a+b
Ck	0.23±0.04[a]	0.10±0.00[a]	0.33±0.03[a]
CkDB2	0.50±0.17[b]	0.26±0.06[b]	0.76±0.22[b]
CkDB5	0.54±0.04[b]	0.26±0.05[b]	0.80±0.09[b]

All values were Means±Standard Error of three replicate cultures, values in the same column with different letters were different at 5% significant level, Chl a: Chlorophyll a and Chl b: Chlorophyll b

respectively) had higher values than the control (0.23±0.04 mg g^{-1}). Similar results were also observed in Chl b and Chl a+b concentration.

According to the above results, the 2 endophytes had positive effects on root growth of *C. kanehirae* cuttings. Many studies also demonstrated similar results[13–17]. Among these treatments, CkDB5-inoculation had increased root dry weight by more than 200% compared with the control. Similar results were also observed by Berta *et al.*[34,35]. Hence, CkDB5-inoculation had higher Mycorrhizal Dependency (MD) compared with CkDB2-inoculation (54 vs. 47%, respectively).

The mycorrhizal seedlings demonstrated that they could absorb more water and nutrients from the soil[36-39] and had higher survival rate and growth rate of more than 100% compared with control seedlings[34,35].

In plants, 90% of biomass (dry weight) came from photosynthesis[40]. While, previous findings indicated that inoculation had no positive effect on number and size of leaves[17] and could not promote more efficient photosynthesis[41,42]. Zhang *et al.*[26] demonstrated that number and area of leaves as well as chlorophyll concentration were all significantly affected by inoculation.

CONCLUSION

The results of this study confirmed the association of the two strains, CkDB2 and CkDB5 with the roots of *C. kanehirae* cuttings. Furthermore, these 2 endophytes could form microsclerotia of DSE with *C. kanehirae* cuttings. They also promoted increase in biomass, leaf area, chlorophyll concentration and net growth of height. *Antrodia camphorata*, restricting to the moribund heart wood of Stout camphor trees is a well-known medicinal polypore fund only in *Cinnamomum kanehirae*. Currently, *C. kanehirae* cuttings are used as a major propagation for plantation. However, these cuttings often fails to survive. The main reason for such failure to occur is due to the lack of axial roots. Hence, CkDB2 and CkDB5 can reasonably be inferred to have the potential to benefit *C. kanehirae* forestation, however CkDB5 has a slightly better performance in promoting growth of *C. kanehirae* cuttings.

ACKNOWLEDGMENT

The author appreciates the effort and assistance of the members of Tree Mycorrhiza Laboratory in National Chiayi University and Chiayi Forest District Office.

REFERENCES

1. Liao, J.C., 1996. *Cinnamomum*. In: Flora of Taiwan, 2nd Edn., Vol. 2, Editorial Committee of Flora of Taiwan (Eds.). Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, pp: 437-448.
2. Chang, T.T. and W.R. Wang, 2005. Basidiomatal formation of *Antrodia cinnamomea* on artificial agar media. Bot. Bull. Acad. Sin., 46: 151-154.
3. Chang, S.H., C.K. Ho and J.Y. Tsay, 2002. *In vitro* culture of *Cinnamomum kanehirae* Hay. Taiwan J. For. Sci., 17: 491-501, (In Chinese).
4. Lin, L.C., T.P. Chang, S.L. Hong, C.K. Hsieh, J.L. Chen and T.Y. Tseng, 2015. The compatibility of *Cinnamomum kanehirae* cuttings and its root fungal endophytes. Quart. J. Chin. For., 48: 127-136, (In Chinese).
5. Kao, Y.P. and S.G. Huang, 1993. Cuttings propagation of *Cinnamomum kanehirae* hag. Taiwan J. For. Sci., 8: 371-388, (In Chinese).
6. Yang, J.C., 2013. Seed germination and silviculture of *Cinnamomum kanehirae*. For. Res. Newslett., 20: 30-32, (In Chinese).
7. Cairney, J.W.G. and A. A. Meharg, 2003. Ericoid mycorrhiza: A partnership that exploits harsh edaphic conditions. Eur. J. Soil Sci., 54: 735-740.
8. Schmid, E., F. Oberwinkler and L.D. Gomez, 1995. Light and electron microscopy of a host-fungus interaction in the roots of some epiphytic ferns from Costa Rica. Can. J. Bot., 73: 991-996.
9. Azcon-Aguilar, C. and J.M. Barea, 1997. Applying mycorrhiza biotechnology to horticulture: Significance and potentials. Sci. Hortic., 68: 1-24.
10. Gibson, B.R. and D.T. Mitchell, 2005. Influence of pH on copper and zinc sensitivity of ericoid mycobionts *in vitro*. Mycorrhiza, 15: 231-234.
11. Lin, L.C., M.J. Lee and J.L. Chen, 2011. Decomposition of organic matter by the ericoid mycorrhizal endophytes of Formosan rhododendron (*Rhododendron formosanum* Hemsl.). Mycorrhiza, 21: 331-339.
12. Douds, Jr.D.D., G. Becard, P.E. Pfeffer, L.W. Doner, T.J. Dymant and W.M. Kayser, 1995. Effect of vesicular-arbuscular mycorrhizal fungi on rooting of *Sciadopitys verticillata* Sieb & Zucc. cuttings. HortScience, 30: 133-134.
13. Scagel, C.F., 2004. Changes in cutting composition during early stages of adventitious rooting of miniature rose altered by inoculation with arbuscular mycorrhizal fungi. J. Am. Soc. Hortic. Sci., 129: 624-634.
14. Scagel, C.F., 2004. Enhanced rooting of kinnikinnick cuttings using mycorrhizal fungi in rooting substrate. HortTechnology, 14: 355-363.
15. Scagel, C.F., 2005. Isolate-specific rooting responses of *Leucothoe fontanesiana* cuttings to inoculation with ericoid mycorrhizal fungi. J. Hortic. Sci. Biotechnol., 80: 254-262.
16. Scagel, C.F., 2005. Inoculation with ericoid mycorrhizal fungi alters fertilizer use of highbush blueberry cultivars. HortScience, 40: 786-794.
17. Oseni, T.O., N.S. Shongwe and M.T. Masarirambi, 2010. Effect of Arbuscular Mycorrhiza (AM) inoculation on the performance of tomato nursery seedlings in vermiculite. Int. J. Agric. Biol., 12: 789-792.
18. Chang, T.T. and W.R. Wang, 2008. The role of four essential oils on mycelial growth and basidiomatal formation of *Antrodia cinnamomea*. Taiwan J. For. Sci., 23: 105-110, (In Chinese).
19. Liao, P.C., D.C. Kuo, C.C. Lin, K.C. Ho, T.P. Lin and S.Y. Hwang, 2010. Historical spatial range expansion and a very recent bottleneck of *Cinnamomum kanehirae* Hay.(Lauraceae) in Taiwan inferred from nuclear genes. BMC Evol. Biol., Vol. 10. 10.1186/1471-2148-10-124
20. Hong, S.L., T.C. Lin and L.C. Lin, 2014. Preliminary study on morphology of root-fungus association of *Cinnamomum kanehirai* at Dabang area. Quart J. Chin. Forest., 47: 393-398, (In Chinese).
21. Chang, C.Y., 2005. Effect of indole-3-butyric acid on the formation of adventitious roots in *Cinnamomum kanehirae* cuttings. Master's Thesis, Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan.
22. Cho, H.Y., C.Y. Chang, L.C. Huang, J.B. Tsai and Z.H. Liu, 2011. Indole-3-butyric acid suppresses the activity of peroxidase while inducing adventitious roots in *Cinnamomum kanehirae*. Bot. Stud., 52: 153-160.
23. Chen, Y.C., 2013. Inoculation effects of arbuscular mycorrhizal fungi on the cutting propagation and resistance against black rot of *Cinnamomum kanehirae* Hayata. Master's Thesis, Department of Forestry and Natural Resources, National Ilan University, Ilan, Taiwan, (In Chinese).
24. Lee, C.F., 2013. Effects of arbuscular mycorrhizal fungi on growth and physiological characteristics of *Cinnamomum kanehirae* cuttings. Master's Thesis, Department of Forestry and Natural Resources, National Chiayi University, Chiayi, Taiwan, (In Chinese).
25. Varma, A., S. Verma, Sudha, N. Sahay, B. Butehorn and P. Franken, 1999. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Applied Environ. Microbiol., 65: 2741-2744.
26. Zhang, H.H., M. Tang, H. Chen and Y.J. Wang, 2012. Effects of a dark-septate endophytic isolate LBF-2 on the medicinal plant *Lycium barbarum* L. J. Microbiol., 50: 91-96.

27. McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild and J.A. Swan, 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol., 115: 495-501.
28. Plenchette, C., J.A. Fortin and V. Furlan, 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil, 70: 199-209.
29. Porra, R.J., 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b* Photosynth. Res., 73: 149-156.
30. Barrow, J. and R. Aaltonen, 2001. Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and the influence of host physiological activity. Mycorrhiza, 11: 199-205.
31. Barrow, J.R., 2003. Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. Mycorrhiza, 13: 239-247.
32. Peterson, R.L., H.B. Massicotte and L.H. Melville, 2004. Mycorrhizas: Anatomy and Cell Biology. NCR Research Press, Ottawa, Canada, Pages: 173.
33. Mathew, A. and R.M. Malathy, 2008. The evidence of mycorrhizal fungi and dark septate endophytes in roots of *Chlorophytum borivillianum*. Acta Botanica Croatica, 67: 91-96.
34. Berta, G., A. Fusconi and A. Trotta, 1993. VA mycorrhizal infection and the morphology and function of root systems. Environ. Exp. Bot., 33: 159-173.
35. Berta, G., A. Trott, A. Fusconi, J.E. Hooker and M. Munro *et al*., 1995. Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*. Tree Physiol., 5: 281-293.
36. Verkade, S.D. and D.F. Hamilton, 1987. Effect of endomycorrhizal inoculum on root initiation and development of *Viburnum dentatum* L. cuttings. J. Environ. Hort., 5: 80-81.
37. Gross, E., L.I.T. Casagrande and F.H. Caetano, 2004. Ultrastructural study of ectomycorrhizas on *Pinus caribaea* Morelet. var. *hondurensis* Barr. and Golf. seedlings. Acta Botanica Brasilica, 18: 1-7.
38. Herrmann, S., R. Oelmuller and F. Buscot, 2004. Manipulation of the onset of ectomycorrhiza formation by indole-3-acetic acid, activated charcoal or relative humidity in the association between oak microcuttings and *Piloderma croceum*: Influence on plant development and photosynthesis. J. Plant Physiol., 161: 509-517.
39. Simard, S.W. and D.M. Durall, 2004. Mycorrhizal networks: A review of their extent, function and importance. Can. J. Bot., 82: 1140-1165.
40. Sage, R.F., M. Li and R.K. Monson, 1999. The Taxonomic Distribution of C_4 Photosynthesis. In: C_4 Plant Biology, Sage, R.F. and R.K. Monson (Eds.). Academic Press, San Diego, California, USA., ISBN: 9780126144406, pp: 551- 584.
41. Marks, S. and K. Clay, 1996. Physiological responses of *Festuca arundinacea* to fungal endophyte infection. New Phytol., 133: 727-733.
42. Wu, L.Q., Y.L. Lv, Z.X. Meng, J. Chen and S.X. Guo, 2010. The promoting role of an isolate of dark-septate fungus on its host plant *Saussurea involucrata* Kar. et Kir. Mycorrhiza, 20: 127-135.