

Lei MA, Xiaoqin WU, Ling ZHENG

## Relationship between plant hormone level excreted by ectomycorrhizal fungi and growth of poplar NL-895

© Higher Education Press and Springer-Verlag 2009

**Abstract** To explore the effects of plant hormones levels excreted by ectomycorrhizal (ECM) fungi on the growth of poplars, *Populus × euramericana* cv. NL-895 seedlings were inoculated with nine species of ECM fungi. We investigated the status of ectomycorrhizal formation and the effects of these fungi on poplar growth, and using the HPLC method, we measured the contents of four kinds of plant hormones, indole acetic acid (IAA), zeatin (Z), gibberellin (GA) and abscisic acid (ABA) in both the culture filtrate and the mycelium of these fungi. The results showed that the effects of nine ECM fungi on the growth of poplar NL-895 varied. The inoculated seedlings, whether or not obvious mycorrhizas were developed, grew better than those non-inoculated ones. All nine ectomycorrhizal fungi excreted the four plant hormones, but at different levels. The hormone contents in culture filtrate were higher than that in mycelium, which showed a definite relationship with poplar growth. Significantly, correlation analysis suggested the height and stem diameter of the poplar were positively correlated with zeatin contents in the mycelium, and were negatively correlated with the levels of ABA or IAA in the mycelium.

**Keywords** ectomycorrhizal fungi, plant hormone, poplar NL-895, HPLC

### 1 Introduction

Mycorrhiza is a mutualistic association formed by higher plants and mycorrhizal fungi. As an important component of nutrient circulation in the natural ecological system, mycorrhiza plays a key role in nutrient absorption and plant growth (Smith and Read, 1997). Mycorrhizal fungi

absorb needed nutrients and other substances from the host plant, and in return, the plant can obtain more water, phosphorus and some other substances from those fungi. Meanwhile, during the growth of fungi themselves and the combination formation with host plant, they excrete some hormones, such as cell growth hormone, cytokinin, vitamin B and indole acetic acid (IAA), to stimulate plant growth.

Previous studies indicated that both IAA (Baser et al., 1987) and cell growth hormone (Simpson, 1974) might alter the morphology of root system. The former helped to modify the cell wall structure of root cortex and mycorrhizal fungi (Durand et al., 1992), to form a close interface between the two, and the latter improved permeability of the membrane of the interface (Craft and Miller, 1974). Different mycorrhizal fungi species, even various strains of the same species, had different abilities in secreting hormones. Ho et al. measured the levels of IAA and cytokinin excreted by 53 strains of *Rhizopogon*, to find that they showed great differences (Chen, 1978).

In this study, we studied the mycorrhizal formation between the nine species of ECM fungi and poplar NL-895, and measured hormone contents of the fungi cultured in vitro. We tried to explore the effects of hormone levels on poplar growth, and to supply some references for revealing the mechanism by which these ECM fungi interact with their host plant.

### 2 Materials and methods

#### 2.1 Ectomycorrhizal fungi

The nine ECM fungi used were *Pisolithus tinctorius* (Pt<sub>2</sub>), *Boletus speciosus* (Bs), *Boletus edulis* (Be), *Laccaria amethystea* (La), *Rhizopogon luteous* (Rl), *Scleroderma luteus* (Sl), *Leccinum scabrum* (Ls), *Xrocomus chrysentero* (Xc) and *Calvatia craniiformis* (Cc). The first eight fungi originated from southwest and northeast China and are preserved in the pathology laboratory of the Nanjing Forestry University. The last fungus was collected from the

Translated from *Scientia Silvae Sinicae*, 2008, 44(7): 43–49 [译自: 林业科学]

Lei MA, Xiaoqin WU (✉), Ling ZHENG  
College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037, China  
E-mail: xqwu@njfu.edu.cn

Nanjing Purple Mountain. These fungi were cultivated in petri dishes for about one week at 25°C.

## 2.2 Ectomycorrhizal formation and poplar growth measurement

The tested poplar, *Populus × euramericana* cv. NL-895, was cultured by the leaf disc method with leaf explants. Grown for 5 months, the seedlings were transplanted after seedling adaptation for 48 h under shade. The growth media used were of two kinds: a non-sterilized soil and a sterilized substrate, a mixture of sand, peat, and vermiculite (1:1:1; v/v/v), sterilized for 2 h. Twenty days after transplant, the seedlings were inoculated with nine ECM fungi by the method of drilling and injecting liquid inoculum, and the control seedlings were added with the same liquid culture medium but without any fungi. At harvest, six months after inoculation, we measured the lengths, stem diameters and mycorrhizal infection rates of all seedlings (Ma and Wu, 2007).

## 2.3 ECM fungi culture *in vitro*

ECM fungi were cultured *in vitro* by the shaking cultivation method. Every 100 mL MMN liquid medium was added into the 250-mL culture flasks and sterilized for 25 min. The fungi were inoculated from petri dishes into the flasks with the hole puncher of 7 mm, and then incubated on vibrating beds (Thermoforma, USA) with the shaking speed of 120 r/min at 25°C for one month. There were six replications for each treatment.

## 2.4 Extraction of the hormones excreted by ECM fungi

The mycelia of the nine ECM fungi cultured in flasks were obtained through vacuum filtration, washed with sterile water, and then dried with filter paper. These mycelia were put into the mortars with 5 mL 80% ice methanol, abraded for 10 min, then transferred to 100 mL conical beakers. The beakers were added with 20 mL 80% ice methanol, shaken up, then put for 40 h at 0°C, shaken at intervals. After filtration, the filtrate was concentrated to 10 mL under reduced pressure. The supernatant fluid was used for measuring the hormone content in the mycelium.

The liquid medium after the mycelium was taken out, together with the lotion for washing mycelium, were concentrated to 10 mL under reduced pressure as the analytical liquid for measuring the hormone contents in culture filtrate.

## 2.5 Measurement of the hormones excreted by ECM fungi

Using HPLC (Agilent 1100 series, USA), the contents of IAA, GA, Z and ABA in the culture filtrate and mycelium samples were measured. The main parameters were as follows: chromatographic column: ZORBAX Eclipse

XDB-C18, analytical 4.6×150 mm (USA); mobile phase: 20% CH<sub>3</sub>CN, 20% CH<sub>3</sub>OH, 60% H<sub>2</sub>O, adjusting the pH value to 3.0 with H<sub>3</sub>PO<sub>4</sub>; velocity of flow: 0.7 mL/min; detector: UV 254 nm×0.1 AuFg.

By HPLC analysis, the standard curve equations for the four hormones were as follows:

$$\text{IAA amt} = (\text{height} + 0.0251454)/0.0578934$$

$$\text{GA amt} = (\text{height} - 0.0797028)/0.03123771$$

$$\text{Z amt} = (\text{height} - 3.39)/0.104$$

$$\text{ABA amt} = (\text{height} + 0.2102242)/0.17457475$$

In the above equations, amt means concentration [ $\mu\text{g}/(100 \text{ mL})$ ], height means the peak height.

The separation chromatography of the tested samples were qualified by retention time, and quantified by the peak height. Data analysis was done with SPSS. Combined with the standard curve equations, we calculated the hormone contents of each treatment (Wu and Bi, 1994; Gong, 1997; Wang et al., 1997; Han et al., 2005).

## 2.6 Statistical analysis

The data on the hormone contents of each group and growth indices of the seedlings were analyzed by SAS software.

# 3 Results

## 3.1 Effects of ECM fungi on the growth of poplar NL-895

After six months of growth, the inoculated seedlings of NL-895 showed definite increases in height and stem diameter compared to the control (Fig. 1). The seedlings inoculated with R1 had the maximum increase of over 70% in height growth. Ls, Sl, Pt<sub>2</sub>, Bs, Cc and Be also had better effects on height, while Xc and La showed weaker effects. On the stem diameter growth, Ls had the best effect with an average increase of 0.15 cm, followed by Sl, R1, Bs, Cc and Pt<sub>2</sub>. Be, La and Xc had some promoting effects too.

Among the nine ECM fungi, Xc, Be, Pt<sub>2</sub>, and La formed clear ectomycorrhizal associations with poplar NL-895. Mycorrhizal infection rates with the four fungi were 68%, 60%, 70% and 20% in non-sterilized soil, and 86%, 80%, 77% and 44% in sterilized substrate, respectively. The other five species did not develop obvious mycorrhizal structures but still promoted poplar growth both in height and stem diameter. It was suggested that the ECM fungi interacted with the poplar seedlings not only in structural changes, but also in the metabolization of rhizospheric environment.

## 3.2 Abilities of excreting hormones

The nine ECM fungi showed significant differences in hormone levels in the culture filtrate and in the mycelium (Fig. 2).

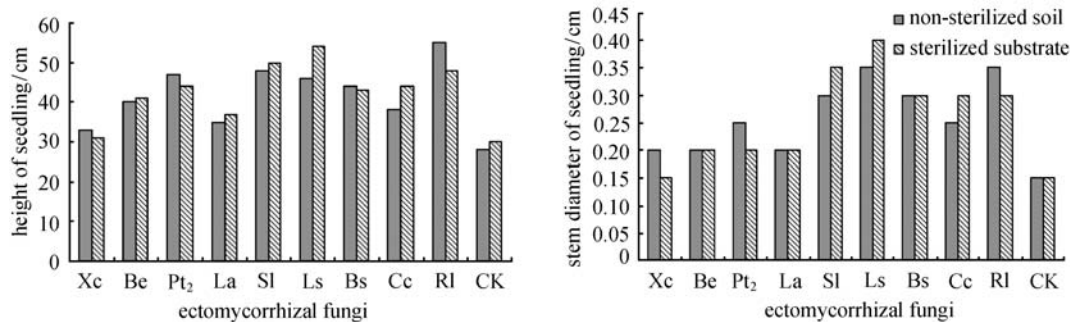


Fig. 1 Effects of nine ectomycorrhizal fungi on growth of poplars

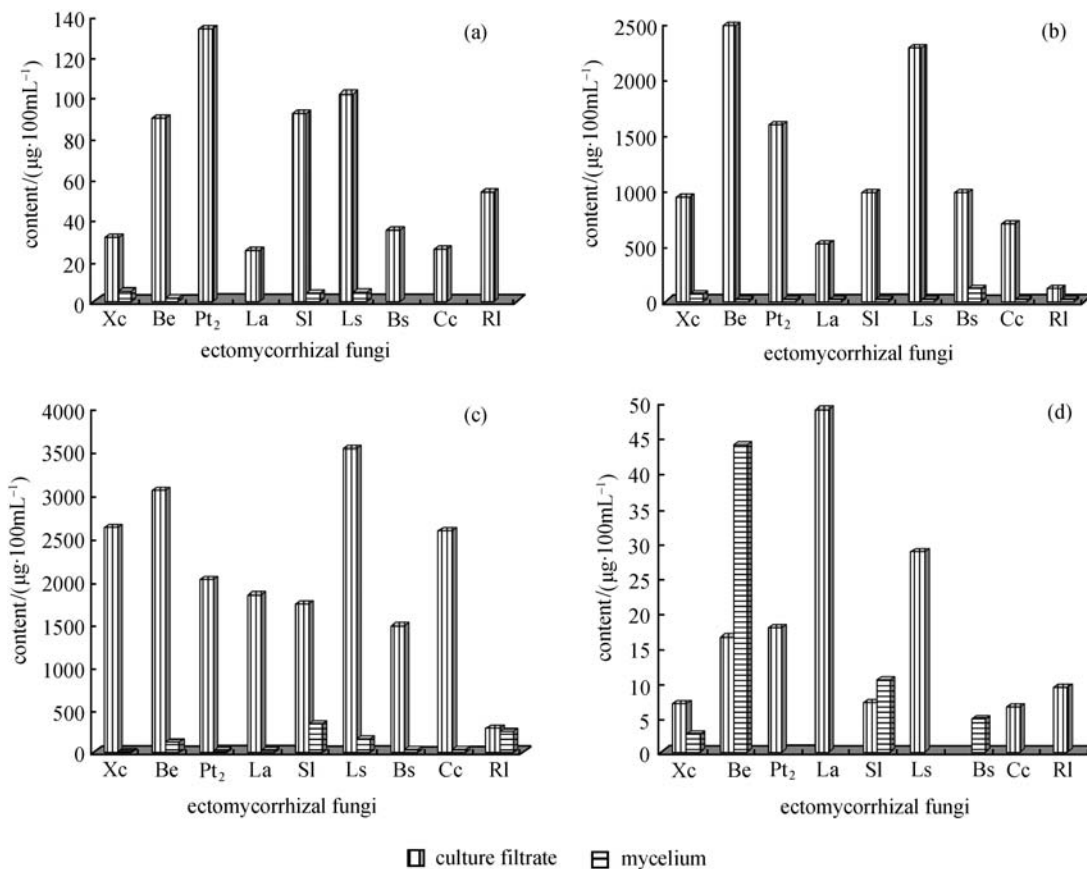


Fig. 2 Comparison of plant hormones secreted by nine kinds of ectomycorrhizal fungi  
a: indole-acetic acid (IAA); b: gibberellin (GA); c: zeatin (Z); d: abscisic acid (ABA)

IAA is an important plant hormone involved in elongation growth. All of nine ECM fungi excreted IAA, with obviously higher contents in the culture filtrate than in the mycelium. The contents were highest in the culture filtrate of Pt<sub>2</sub> and Ls, over 100  $\mu\text{g}/(100\text{ mL})$ , followed by Sl, Be, and RI, and the contents were lower in Bs, Xc, Cc and La by about 20 to 30  $\mu\text{g}/(100\text{ mL})$ . The mycelium had rather lower levels of IAA, which were not detected in RI and Pt<sub>2</sub> groups (Fig. 2(a)).

GA mainly helps to break dormancy and accelerate

growth. It was shown in Fig. 2(b) that nine ECM fungi had higher GA levels in culture filtrate, with the average content of 1172.4  $\mu\text{g}/(100\text{ mL})$ . The content reached 2277.3  $\mu\text{g}/(100\text{ mL})$  in Be and 2473.0  $\mu\text{g}/(100\text{ mL})$  in the Ls treatments, and the lowest content was 116.1  $\mu\text{g}/(100\text{ mL})$  in the RI filtrate. While in the mycelium, the GA levels were very low, with the average content of 33.5  $\mu\text{g}/(100\text{ mL})$ . Except for Xc and Bs, the other six fungi had lower contents, ranging from 12.2 to 20.9  $\mu\text{g}/(100\text{ mL})$ .

Zeatin plays a major role in cell division and

differentiation. Among the four hormones excreted by the ECM fungi, zeatin showed the highest content, which was higher in the culture filtrate than in the mycelium. Among the nine fungi, Ls had the highest content of about 3500 µg/(100 mL) in culture filtrate, R1 had the lowest of 277.7 µg/(100 mL), and the others had the contents ranging from 277.7 to 3036.7 µg/(100 mL). In the mycelium, Sl, R1 and Ls had relatively higher zeatin levels, with the maximum content of 326.4 µg/(100 mL), while the other fungi had the only average content of 56.6 µg/(100 mL) (Fig. 2(c)).

ABA has inconsistent effects on plant growth. A lower concentration of ABA stimulates rooting and hypocotyl elongation, while a higher concentration inhibits the growth of stem, root, coleoptile and leaf. In our study, the ABA level was much lower than the other three hormones both in culture filtrate and in mycelium. The highest content was for La, but less than 50 µg/(100 mL), and the other contents varied from 17.8 to 43.9 µg/(100 mL) (Fig. 2(d)).

Many previous researches have indicated that ABA acted as the natural antagonist of GA, and the two shared the same precursor for synthesis (Han et al., 2005). From Figs. 2(b) and 2(d), we found that it presented an ebb and flow relationship of hormone levels between ABA and GA.

### 3.3 Relationship between hormone level and poplar growth

We made the correlation and regression analysis of hormone contents and height and stem diameter growth

of the seedlings, and found that hormone contents showed various correlations with poplar growth between the different ECM fungi. Significantly, the height of the seedling was positively correlated with the stem diameter, IAA content in the culture filtrate, as well as the zeatin content in the mycelium, while the stem diameter was positively correlated with the height and zeatin content in the mycelium (Table 1).

Interaction between the hormones and their effects on poplar growth were very complicated, so by multiple linear regression analysis, we further explored the relationship between the hormone contents and poplar growth.

Multiple linear regression analysis of the height showed the regression model was significant with the related coefficient (*R*) of 0.948 (coefficient of determination  $R^2 = 0.898$ ), *F*-value of 12.135, *P*-value of 0.000 in the regression equation (Table 2).

As to the standard regression coefficient (Table 3), the zeatin content in the mycelium had the largest absolute value (0.982) of standard regression coefficient, as well as of the partial correlation coefficient. The zeatin level showed a significant positive correlation with height (*P* = 0.000), which indicated that it had the best impact on height growth. ABA and IAA contents in the mycelium showed very notable negative correlations with height (*P* < 0.01).

Multiple linear regression analysis of stem diameter showed the regression model was significant with the related coefficient (*R*) of 0.964 (coefficient of determination  $R^2 = 0.929$ ), *F*-value of 17.904, and *P*-value of 0.000 (Table 4).

**Table 1** Correlations between the four plant hormones and the height and diameter growth of NL-895 poplar

	height	stem diameter	filtrate IAA	mycelium IAA	filtrate GA	mycelium GA	filtrate Z	mycelium Z	filtrate ABA	mycelium ABA
height	1	0.893**	0.645**	0.084	0.310	-0.076	0.146	0.709**	0.059	0.000
stem diameter	0.893**	1	0.387	0.222	0.207	0.039	0.183	0.656**	0.003	-0.175
filtrate IAA	0.645**	0.387	1	0.275	0.735**	-0.185	0.456*	0.421	0.206	0.289
mycelium IAA	0.084	0.222	0.275	1	0.424	0.096	0.559*	0.317	0.029	0.076
filtrate GA	0.310	0.207	0.735**	0.424	1	0.022	0.824**	0.107	0.259	0.593**
mycelium GA	-0.076	0.039	-0.185	0.096	0.022	1	0.069	-0.257	-0.320	-0.031
filtrate Z	0.146	0.183	0.456*	0.559*	0.824**	0.069	1	-0.047	0.367	0.344
mycelium Z	0.709**	0.656**	0.421	0.317	0.107	-0.257	-0.047	1	-0.077	0.215
filtrate ABA	0.059	0.003	0.206	0.029	0.259	-0.320	0.367	-0.077	1	-0.031
mycelium ABA	0.000	-0.175	0.289	0.076	0.593**	-0.031	0.344	0.215	-0.031	1

**Table 2** Variance analysis of the height model of NL-895 poplar

	sum of squares	df	mean square	<i>F</i>	<i>P</i>	<i>R</i>	$R^2$
regression	1023.256	8	127.907	12.135	0.000	0.948	0.898
residual	115.944	11	10.540				
total	1139.200	19					

**Table 3** Coefficients between four plant hormones and the height model of NL-895 poplar

	Non-standardized coefficient		standardized coefficient	<i>t</i>	<i>P</i>	partial correlation coefficient
	<i>B</i>	standard error	$\beta$			
constant	29.932	2.223		13.463	0.000	
filtrate IAA	0.016	0.042	0.088	0.387	0.706	0.116
mycelium IAA	-2.187	0.535	-0.593	-4.090	0.002	-0.777
filtrate GA	0.006	0.004	0.580	1.554	0.148	0.424
mycelium GA	0.041	0.025	0.184	1.645	0.128	0.444
filtrate Z	0.002	0.002	0.224	0.950	0.362	0.275
mycelium Z	0.069	0.010	0.982	6.611	0.000	0.894
filtrate ABA	-0.031	0.063	-0.058	-0.492	0.632	-0.147
mycelium ABA	-0.354	0.098	-0.609	-3.606	0.004	-0.736

**Table 4** Variance analysis of the diameter model of NL-895 poplar

	sum of squares	<i>df</i>	mean square	<i>F</i>	<i>P</i>	<i>R</i>	<i>R</i> <sup>2</sup>
regression	0.102	8	0.013	17.904	0.000	0.964	0.929
residual	0.008	11	0.001				
total	0.109	19					

**Table 5** Coefficients between the four plant hormones and the diameter model of NL-895 poplar

	unstandardized coefficient		standardized coefficient	<i>t</i>	<i>P</i>	partial correlation coefficient
	<i>B</i>	standard error	$\beta$			
constant	0.153	0.018		8.391	0.000	
filtrate IAA	-0.001	0.000	-0.647	-3.405	0.006	-0.716
mycelium IAA	-0.021	0.004	-0.589	-4.852	0.001	-0.826
filtrate GA	0.0001	0.000	1.271	4.067	0.002	0.775
mycelium GA	0.0004	0.000	0.180	1.927	0.080	0.502
filtrate Z	0.000015	0.000	0.212	1.075	0.305	0.308
mycelium Z	0.001	0.000	1.249	10.043	0.000	0.950
filtrate ABA	-0.001	0.001	-0.132	-1.336	0.209	-0.374
mycelium ABA	-0.006	0.001	-1.037	-7.340	0.000	-0.911

About the standard regression coefficient (Table 5), GA content in culture filtrate had the largest absolute value (1.271), which had the best effects on the stem diameter, followed by the zeatin content in the mycelium (1.249). Both showed very notable positive correlations with the stem diameter ( $P < 0.01$ ), while the contents of the ABA in the mycelium, IAA in the culture filtrate and in the mycelium showed very notable negative correlations with the stem diameter ( $P < 0.01$ ).

The results suggested that the growth-promoting effect on poplar seedlings was not due to a certain major hormone, but rested with the dynamic equilibrium among those hormones excreted by the ECM fungi.

## 4 Conclusions and discussion

All the tested ECM fungi boosted poplar growth to different degrees. The fungi excreted plant hormones at various levels, and the contents were significantly higher in the culture filtrate than in the mycelium. IAA was not detected in the mycelium of five fungi among the nine. It was indicated that the hormones excreted by the ECM fungi were released out of the hyphae cells, and were absorbed by the host plant through mycorrhizal association (Wang et al., 1997).

Correlation analysis indicated a positive correlation between the zeatin levels in the mycelium and poplar

growth, while showed negative correlations between IAA and ABA contents in the mycelium and poplar growth. Among the five fungi with higher hormone levels, Be, Xc, and Pt<sub>2</sub> had good symbiotic relationships with poplar. It showed that those hormones excreted by ECM fungi acted as direct signal molecules or indirect messengers for the mutual recognition of the two parts at the early stage of mycorrhizal formation. The other fungi with lower hormone levels were not found to form obvious ectomycorrhizas, but they all promoted poplar growth.

Levisohn (1953) inoculated *Chamaecyparis lawsoniana* and *Thuja occidentalis* with ECM fungi to find that the fungi had distinct effects on host plant growth though without ectomycorrhizal formation, which showed that the growth hormone was not produced after the symbiotic formation. Furthermore, the five fungi, Sl, Ls, Bs, Cc and Rl developed a few ectomycorrhizas in non-sterilized soil where no mycorrhizal formation in control seedlings and no ectomycorrhizas were formed in the sterilized substrate. It might be that some rhizosphere microorganism in the non-sterilized soil interacted with these fungi and promoted mycorrhizal formation.

In conclusion, the growth-promoting effect of mycorrhizal fungi, on one hand, was due to mycorrhizal formation to enlarge absorption areas, and on the other hand, was owed to the coordination of the inner and exogenous hormones excreted by the fungi acting on plant roots, or to the improvement of rhizospheric environment. Additionally, for the consistency of the experiment, all ECM fungi were grown under the same conditions, such as the temperature of 25°C, which might not be optimum for all fungi. Therefore, the hormone levels were possibly correlated with the culture conditions. It needed further research on how the internal hormone levels of host plant changed after inoculation.

The contents of four hormones in the culture filtrate and in the mycelium of Rl, Ls and Sl, which had better effects on height and diameter growth, were different. Notably, the three fungi had much higher contents of zeatin in the mycelium than other fungi. As a major internal cytokinin that can be transferred (Brenner and Chezhk, 1995), zeatin is synthesized mainly at the root tip (Zhou and Wei, 2006), where the interaction between mycorrhizal fungi and plant root occurs. Thus, cytokinin excreted by ECM fungi might be transferred from the root tip to the overground parts of plant, to have physiological effects. In future studies, we will be more concerned about the effects of zeatin on poplar growth.

**Acknowledgments** This study was financially supported by the National Natural Science Foundation of China (Grant No. 30571471) and the Natural Science Foundation of Jiangsu Province, China (No. BK2004217). We are grateful to Professor Zongming HE from the Fujian Agriculture and Forestry University for his help in data analysis, and to the Bioengineering Laboratory of the Nanjing Forestry University for hormone measurements.

## References

- Baser C M, Garrett J E, Mitchell R J (1987). Indolebutyric acid and ectomycorrhizal inoculation increase lateral root initiation and development on container-grown black oak seedlings. *Can J For Res*, 17: 36–39
- Beyrle H (1995). The role of phytohormones in function and biology of mycorrhizas. In: Varma A, Hock B, eds. *Mycorrhizas: Structure, Function, Molecular Biology*. Berlin: Springer-Verlag, 365–390
- Brenner M L, Chezhk N (1995). The role of hormones in photosynthate partitioning and seed filling. In: Davies P J, ed. *Plant Hormones*. Netherlands: Kluwer Academic Publishers, 649–670
- Chen Y M (1978). Effects of mycorrhizal fungi on the growth of poplars. *Compilation of reference material for exotic pines*, 10: 16–24
- Craft C B, Miller C O (1974). Detection and identification of cytokinins produced by mycorrhizal fungi. *Plant Physiol*, 53: 586–588
- Durand N, Deband J C, Casselton L A (1992). Isolation and preliminary characterization of 5-fluorindole-resistant and IAA-overproducer mutants of the ectomycorrhizal fungus *Hebeloma cylindrosporum* Romagnesi. *New Phytol*, 121: 545–553
- Fu L, Wang J M, Wang M L (1998). Measuring method of ABA and 3-IAA in plant tissue. *Shandong Chem Ind*, 5: 59–60 (in Chinese)
- Gong M Q (1997). *Mycorrhizal Research and Application*. Beijing: China Forestry Publishing House (in Chinese)
- Han L J, Fan X, Yuan Z H (2005). Isolation and estimation of auxin from marine algae. *Oceanol Et Limnol Sin*, 36(2): 167–171 (in Chinese)
- Levisohn I (1953). Growth response of tree seedlings to mycorrhizal mycelia in the absence of a mycorrhizal association. *Nature*, 172: 316
- Ma L, Wu X Q (2007). Mycorrhizal formation of nine ectomycorrhizal fungi on poplar cuttings. *J Nanjing For Univ (Nat Sci Ed)*, 31(6): 29–33 (in Chinese)
- Simpson D G (1986). Auxin stimulates lateral root formation of container grown Interior Douglas fir seedlings. *Can J For Res*, 16: 1135–1139
- Smith S E, Read D J (1997). *Mycorrhizal Symbiosis*. London: Academic Press
- Zhou L, Wei Q C (2006). The effect of cytokinins on fruit and seed development. *Plant Physiol Commun*, 42(3): 549–553
- Wang Y Z (1997). Plant hormones production by 4 species ectomycorrhizal fungi in pure culture. *Microbiol*, 24 (2): 72–74 (in Chinese)
- Wu G X, Bi G H (1994). Determination of indole-3-acetic acid and abscisic acid in apple leaves by high-performance liquid chromatography. *J Shandong Agr Univ*, 25(1): 51–55 (in Chinese)