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檀香寄主根际 pH 值对檀香生长及其寄主偏好性的影响

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摘要: 为研究根际 pH 对檀香的生长和寄主偏好性的影响,用便携式酸度计检测了中国科学院华南植物园檀香园及附近的 61 种植物根部的 pH 值,并考察了檀香在不同 pH 梯度下的生长情况。结果表明,几乎所有优良寄主根部的 pH 值均为5.0~6.0;在 pH 5.5 的培养条件下,檀香茎和根的长度明显比在其他 pH 值下的要长。这说明檀香生长的最佳根际 pH 值为 5.5。这有助于对檀香优良寄主进行选择。

关键词: 檀香; 寄主; 根际; pH; 偏好性
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Influence of Rhizospheric pH Value of Host on Growth of Indian Sandalwood and Preference to Host

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Abstract: The effects of rhizospheric pH value of host on growth of Indian sandalwood (*Santalum album* L.) and preference to host were studied. Rhizospheric pH values of 61 hosts of *S. album* were measured by using portable precise acidimeter, and the growth status of *S. album* under a gradient rhizospheric pH were observed. The results showed that rhizospheric pH of almost all fine hosts ranged from 5.0 to 6.0, and the root length and shoot height of *S. album* grown at pH 5.5 were significantly higher than those grown at other pHs. It was suggested that the optimum pH of Indian sandalwood was 5.5. It is helpful to choose a fine host of *S. album*.

Key words: Indian sandalwood; Host; Rhizosphere; pH; Preference

Indian sandalwood, *Santalum album*, is a small tropical evergreen tree of the Santalaceae family. It is indigenous to Indonesia, and was introduced to Indian Peninsula in centruries ago. It is one of the most valuable and widely used essential oil bearing plants in the World. Its heartwood can be used to make high value handicrafts, furnitures and religious objects. The sandalwood oil obtained from sandalwood is very distinctive and is used in countless applications, including cosmetic, perfumery, medical agent and aromatherapy industries, and recently in skin cancer prevention[1-5]. Because of the wide use and tremendous application potential of sandalwood, the price of its heartwood has boomed in recent years, and the auction price exceeds 110000 Australian dollars per tone[6]. Thus, an alternative supply of Indian sandalwood is urgently needed. *S. album* is not a Chinese native plant species; it was introduced to

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China from Indonesia and India in the 1960s and 1980s and is now widely cultivated in South China[7].

The sandalwood tree is a semiparasitic plant. After seedling period, it need be co-cultivated with a host, and invades the tissues of host in order to parasitize water and nutrients through a unique organ called a haustorium. In sandalwood tree cultivation and introduction, a naturally occurring problem is that sandalwood trees show a preference in their choice of host. Co-cultivation pot experiments with a single host species indicated that the growth status of the sandalwood tree varied among different hosts[8–9]. The co-cultivating hosts could be divided into three types: (1) fine host, with which sandalwood trees can grow faster, have dark green leaves, and more haustorial connections and big haustorial diameter; (2) ordinary host, with which sandalwood trees grow very slowly, with thin stems, yellow-green leaves, and few of haustorial connections; (3) non host, with which sandalwood trees stop growing, almost can't establish haustorial connections, the sporadically established haustorial connections are loose, haustoria are wizened, leaves gradually turn yellow and senesce, and trees will die within three months. The previous study confirmed that, compared to ordinary hosts, sandalwood trees co-cultivated with fine hosts had longer stems and roots and more haustorial connections than the other two types[10]. The same experiment performed in South China Botanical Garden showed similar result[11]. Another study on *Thesium chinense*, which is also a semiparasitic plant in the Santalaceae, showed that it also had a host preference[12]. However, the underlying mechanisms are not well understood. Our primary interest was thus to focus on answering why the sandalwood tree prefers to choose its host and how this choice was made. In this study, this obscure preference was discussed through a couple of experiments, it was helpful to understanding the semiparasitic behaviour of *Santalum album*.

1 Materials and methods

1.1 Measurement of host rhizospheric pH value

Santalum album, which was introduced to South China since 1962, is now only preserved in the Sandalwood Garden of South China Botanical Garden, Chinese Academy of Science. The rhizospheric pH values of 61 species, including fine hosts, ordinary hosts and non hosts grown in or around the Sandalwood Garden, were measured using an HI 99121 Portable Soil pH meter (HANNA instruments). The measured rhizosphere locates in the middle of sandalwood tree and the host, about 30 cm below the surface. The measurement for each host was replicated at least 3 times. The means and standard deviations were plotted by SPSS 19 Software. The rhizospheric pH values of fine hosts, ordinary and non hosts were compared by an independent sample *t*-test.

1.2 Culture of sandalwood seedlings

The seeds of sandalwood tree were imported from India, and then soaked in germination promoting solution containing 500 – 800 mg L^{-1} GA_3 (gibberellic acid) (Shanghai Lanshen Biochemical production) for 24 hours. The soaked seeds were sowed under yellow sand surface 3–5 cm in greenhouse, and watered every morning one time a day. Seeds would germinate in one month, picked out the seedlings with the same height for further uses.

1.3 Growth of sandalwood seedlings

180 pots (10 cm diameter × 18 cm height) were equally divided into 9 groups, including eight treatment groups and one control group. In each group, 10 pots host *Kuhnia rosmarnifolia* was rooted as the partner, while the other 10 pots were not. The pot medium (1 L for each pot) was generated by well mixing soil∶wood chips∶yellow sand at the ratio of 3∶1∶1. This proportion of components could generate an appropriate rhizospheric condition in which the ratio of reserved water would not too high or too low. Eight treatment groups were separately pre-watered with solutions containing 40 mmol/L dibasic sodium phosphate-citric acid buffer at pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, respectively, one time a day for several days, in order to produce a pH gradient. The control group was watered with water at pH 7.3. When the pot medium pH condition in each treatment group was

closely equal to its corresponding buffer solution, sandalwood seedlings with the same height were transplant in pots, one seedling per pot. Every treatment group was watered with its own pH buffer one time a day, control group just only use water. After six months, the shoot height and root length of plantlets were measured. Differences in growth status among groups were interpreted by analysis of variance (ANOVA).

2 Results

2.1 Rhizospheric pH of 61 species

Rhizospheric pHs of most fine hosts ranged from 5.0 to 6.0 (Table 1), including *Eriobotrya japonica*, *Cyclosorus parasiticus*, *Alstonia scholaris*, *Loropetalum chinense*, *Murraya exotica*, *Callistemon rigidus*, *Psychotria rubra*, *Tephrosia candida*, *Cassia bicapsularis*, *Acacia confusa*, *Litsea glutinosa*, *Jasminum sambac*, *Acacia farnesiana*, *Blechnum orientale*, *Melastoma candidum*, *Lantana camara*, *Mallotus apelta*. All these species are already known as fine hosts according to cultivation experience accumulated over the past 50 years in the South China Botanical Garden[13]. These plants, such as *Rauvolfia vomitoria*, *Acacia auriculiformis*, *Cassia siamea*, *Albizia falcataria*, *Broussonetia papyifera* and *Dracontomelon duperreanum*, are also fine host for sandalwood tree and the rhizospheric pHs are higher than 6.0. Some other plants, such as *Eugenia uniflora*, *Allemanda cathartica*, *Clerodendrum cyrtophyllum*, *Litsea rotundifolia*, *Macaranga tanarius* are ordinary hosts. Sandalwood tree co-cultivating with these hosts grew very slow. Their rhizospheric pHs are all close to 6.0. Independent samples *t*-test showed those rhizospheric pH values of ordinary and none hosts are significantly higher than those of fine hosts at 0.01 level. The data didn't show a absolute co-relation between host type and rhizospheric pH, but told a statistical pattern that rhizspheric pHs of fine hosts tend to range from 5.0 to 6.0, and those of ordinary and none hosts would higher than 6.0. Although there are several exceptions, the pattern is quite obvious.

Table 1 Rhizospheric pH of 61 plant species

Species	Mean	Standard deviation	Number of measurement	Host type
Eriobotrya japonica	5.27	0.06189	6	Fine host
Cyclosorus parasiticus	5.03	0.13357	6	Fine host
Tephrosia candida	5.00	0.16793	4	Fine host
Alstonia scholaris	5.43	0.05586	6	Fine host
Loropetalum chinense	5.17	0.20556	6	Fine host
Murraya exotica	5.64	0.20856	6	Fine host
Callistemon rigidus	6.11	0.07339	6	Fine host
Psychotria rubra	4.89	0.12121	4	Fine host
Cassia bicapsularis	5.12	0.18464	4	Fine host
Acacia confusa	4.93	0.17569	4	Fine host
Litsea glutinosa	5.41	0.12926	8	Fine host
Jasminum sambac	5.22	0.19138	4	Fine host
Acacia farnesiana	5.01	0.22487	4	Fine host
Blechnum orientale	5.61	0.07416	4	Fine host
Melastoma candidum	5.57	0.14361	4	Fine host
Lantana camara	5.57	0.10210	4	Fine host
Mallotus apelta	5.88	0.05909	4	Fine host
Eupatorium odoratum	6.00	0.10231	4	Fine host
Albizia lebbeck	5.54	0.08869	4	Fine host

续表(Continued)

Species	Mean	Standard deviation	Number of measurement	Host type
Broussonetia papyifera	6.53	0.17407	4	Fine host
Cassia siamea	6.39	0.11383	4	Fine host
Rauvolfia vomitoria	6.92	0.17115	4	Fine host
Dracontomelon duperreanum	7.07	0.23922	4	Fine host
Albizia falcataria	6.22	0.10000	4	Fine host
Acacia auriculiformis	6.48	0.17000	4	Fine host
Cassia surattensis	6.57	0.11314	6	Ordinary or none host
Ficus virens	6.81	0.10211	6	Ordinary or none host
Zingiber officinale	7.14	0.15113	6	Ordinary or none host
Bauhinia variegata	7.28	0.09331	6	Ordinary or none host
Ochna integerrima	7.40	0.15943	6	Ordinary or none host
Evodia lepta	7.38	0.14063	6	Ordinary or none host
Syzygium cumini	7.74	0.09268	6	Ordinary or none host
Calathea rotundifolia	7.65	0.05785	6	Ordinary or none host
Mangifera indica	7.88	0.07891	6	Ordinary or none host
Citrus maxima	7.97	0.10387	6	Ordinary or none host
Delonix regia	7.88	0.02898	6	Ordinary or none host
Gmelina arborea	6.96	0.21219	4	Ordinary or none host
Eugenia uniflora	5.78	0.24125	4	Ordinary or none host
Alocasia macrorrhiza	6.39	0.18998	4	Ordinary or none host
Allemanda cathartica	6.01	0.09967	4	Ordinary or none host
Clerodendrum cyrtophyllum	6.03	0.08921	4	Ordinary or none host
Litsea rotundifolia	5.96	0.25343	4	Ordinary or none host
Macaranga tanarius	6.00	0.18464	4	Ordinary or none host
Heritiera angustata	6.07	0.16042	4	Ordinary or none host
Syzygium australe	6.45	0.20728	4	Ordinary or none host
Ficus benjamina	6.18	0.27500	4	Ordinary or none host
Brassaia actinophylla	6.62	0.24998	4	Ordinary or none host
Aleurites montana	6.96	0.33116	4	Ordinary or none host
Aleurites moluccana	7.37	0.23977	4	Ordinary or none host
Cinnamomum porrectum	7.10	0.37798	4	Ordinary or none host
Ceratopetalum succirubrum	6.86	0.09416	4	Ordinary or none host
Pseudoweinmannia lachnocarpa	6.69	0.23558	4	Ordinary or none host
Endiandra sankeyana	6.61	0.24878	4	Ordinary or none host
Grevillea baileyana	6.74	0.07326	4	Ordinary or none host
Grevillea robusta	6.72	0.15022	4	Ordinary or none host
Buckinghamia celsissima	6.05	0.11633	4	Ordinary or none host
Xanthostemon chrysanthus	6.29	0.28826	4	Ordinary or none host
Melaleuca bracteata	5.43	0.05795	4	Not defined
Ficus hispida	6.89	0.14213	4	Not defined
Cinnamomum camphora	5.90	0.03559	4	Not defined
Acmena acuminatissima	6.07	0.12606	4	Not defined

2.2 Effect of pH on growth of sandalwood tree

Those sandalwood seedlings that are not co-cultivated with a host gradually died within six months. Hence, all collected data in this paper came from plantlets co-cultivated with a host. The results showed that shoot height and root length of sandalwood seedlings grown in pH 5.5 were the highest among all pH gredients. The difference between shoot height and root length of seedlings in different pH treatments are compared by One-Way ANOVA. The results showed that root length and shoot height are significantly higher ($P = 0.05$) at pH 5.5 than at other pHs by Duncan's multiple range test (Table 2).

Table 2 Effect of pH on growth of sandalwood seedlings

pH	Stem length (cm)	Root length (cm)
3.5	10.63 ± 0.92c	6.13 ± 3.98c
4.0	11.25 ± 1.28bc	7.00 ± 2.07c
4.5	11.88 ± 0.99bc	8.13 ± 1.81bc
5.0	12.13 ± 2.03b	10.88 ± 4.22b
5.5	13.63 ± 1.77a	17.63 ± 5.15a
6.0	10.71 ± 0.76bc	9.00 ± 3.22bc
6.5	11.00 ± 1.00bc	7.56 ± 1.74bc
7.0	10.88 ± 1.13bc	7.13 ± 2.10c
Control	10.70 ± 1.25bc	7.60 ± 2.46bc

Data followed different letters within column indicate significant difference at 0.05 level according to Duncan's multiple range test.

3 Discussion

3.1 Rhizospheric pH condition for haustoria development

The results in this paper showed the difference of rhizospheric pH among fine hosts and ordinary and none hosts. Sandalwood tree prefers a weak acid rhizospheric condition to form semiparasitic connections with its host plants, the optimum pH ranged from 5.0 to 6.0(Table 1). However, there are six fine host species whose rhizospheric pH values are greater than 6.0, such as *R. vomitoria*, *A. auriculiformis*, *C. siamea*, *A. falcataria*, *B. papyifera* and *D. duperreanum*. *A. auriculiformis*, *C. siamea* and *A. falcataria*. They are Leguminosae family plants that can engage symbiosis with nitrogen-fixing bacteria. Establishing semiparasitic connections with these hosts can guarantee the sandalwood tree a reliable source of nutrition. A previous study reported that *R. vomitoria* and *D. duperreanum* are fine hosts of sandalwood trees[13]. However, from our observation, the root-root connection could not be found between sandalwood tree and these two plants. One reason was that sandalwood tree already established haustorial connections with other neighboring fine host roots, which in fact it does. These connections are enough for guaranteeing sandalwood tree growth, so it does not need to establish a connection with *R. vomitoria* and *D. duperreanum* any longer. Another reason is probably that the rhizospheric pH is not appropriate for sandalwood tree semiparasite.

3.2 Role of acidity on sandalwood semiparasite

The results showed that sandalwood trees grow the best in a weak acidic condition (pH = 5.5), and most fine hosts possess the same rhizospheric pH values. There is no enough evidence to conclude that the ideal plants being fine hosts is just because they provide an optimal rhizospheric pH for sandalwood tree, but there could be some veiled association between these two. A study case demonstrated that during haustorium development, sublocalization of acid phosphatase (ACP) changed[14]. Transcriptome study showed expression level of *ACP* gene were higher during haustorium development stage[15]. The activity of ACP reach up to maximum in a weak acidic condition[16–18]. ACP probably plays as a "switch", mediating sandalwood tree singaling the environment. To solidify this, molecular approach should be introduced.

This study showed correlation between sandalwood preference on choosing its fine host and rhizospheric pH value, it suggested that ACP would play an important role in sandalwood semiparasite behavior. The results provided a reference for choosing fine host of sandalwood tree in cultivation and enlighted the further study on elucidating mechanism on sandalwood semiparasitic behavior.

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