不同居群白木香的染色体研究

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摘 要：采用常规压片法和改良 BSG 法对 3 个居群白木香的染色体核型及 Giemsa C-带带型进行研究。结果表明：3 个居群白木香的核型均属 2B 类型，其中广西居群白木香的核型公式为 2n=16=4m+8sm+4st；其他两个居群白木香的核型公式为 2n=16=6m+6sm+4st，居群间核型变异不明显。白木香的 C 带带型为 CIT 型，具有着丝粒带、中间带、端带和全带。3 个居群白木香 C 带的分布、数目和类型不完全一样，出现了带型的多态性。

关键词：白木香；核型；Giemsa C 带

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Chromosomal studies on populations of *Aquilaria sinensis*

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Abstract: For the first time, a conventional analysis of chromosome karyotype and Giemsa C-banding was carried out in three populations of *Aquilaria sinensis*. Three populations showed same chromosome number of 2n=2x=16 and the asymmetry of the karyotype was categorized as type 2B. The variance of karyotype was not obvious in three populations, only karyotypical formula (2n=16=4m+8sm+4st) in one population was different from the other two (2n=16=6m+6sm+4st). The Giemsa C-banding pattern of *A. sinensis* was CIT pattern. Within Giemsa C-banding pattern of *A. sinensis* there were centromeric bands, intercalary bands, telomeric bands and whole bands. The distributed position, numbers and types of the C-banding on the chromosome of *A. sinensis* had shown the clear polymorphisms.

Key words: *Aquilaria sinensis*; karyotype; Giemsa C-banding

1 Introduction

Aquilaria sinensis (Thymelaeaceae) is one of very few species of tropical trees and is the source of agarwood (Ng *et al.*, 1997), one of the most highly valuable forest products currently traded internationally. Agarwood (also known as aloeswood, eaglewood and gaharu, among many other common names) is a fragrant wood that has been traded since biblical times for use in religious functions and for medicinal and aromatic preparations (Barden *et al.*, 2000).

A. sinensis is almost distributed in Guangdong, Hainan and Guangxi provinces in China. Since the 15th Century, agarwood has been collected and used as a drug in China. Studies revealed that agarwood has remarkable anticancer activity (Gunasekera *et al.*, 1981). Benzene extractable compounds possess potent central nervous system antidepression activities (Okugawa *et al.*, 1993, 1996), and agarwood is considered as new promising nervous system drug (Chen, 1999). High consumer demand, particularly from Middle Eastern and Asian markets, combined with decreasing supply has pushed prices progressively higher to the extent

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that top grade agarwood can sell for over USD 10 000/kg in end-use markets (Barden *et al.*, 2000).

Previous studies of *A. sinensis* have mostly focused on its tissue culture, chemical composition and pharmacological action of agarwood. However, its cytogenetic data remain unknown. In this article, we studied the chromosome karyotype and Giemsa C-banding pattern of *A. sinensis* occurring in different populations, which offered the evidence for further research of identification, breeding and evolution.

2 Materials and methods

2.1 Plant material

Seeds of *A. sinensis* were from Nanning (Guangxi), Maoming (Guangdong), Tunchang (Hainan) and identified by Xing Fu-Wu, professor of South China Botanical Garden in Guangzhou.

2.2 Karyotype analysis

Roots were collected from potted plants of selected genotypes kept in a shade house. Root tips were treated with 0.002 mol/L 8-hydroxyquinoline solution for 2 h at 18—20 ℃, and fixed in Carnoy's I fixative (ethanol : acetic acid = 3 : 1 v/v) for 2 — 24 h. Samples were hydrolysed in 1 mol/L HCl at 60 ℃ for 8—10 min, and digested with 2.5% cellulase enzyme for 30 min at 37 ℃, then squashed in a drop of *Carbol fuchsin* on a microscope slide. With the cover slip in place, the slide was heated and then pressed firmly to flatten cells. All observations were made under oil immersion objective lens using a Olympus (BX41) microscope. Only metaphase cells, in which individual chromosomes were clearly distinguishable, were used for making counts and >10 dividial cells were counted for each sample to determine the chromosome number. Photographs of the 3—5 best individual cells were enlarged for karyotyping.

Take photos after the chromosome were properly stained and select clear photos for analysis. At least three root tip cells per individual were used to determine the karyotype of somatic chromosomes at metaphase. We measured the haploid absolute length and the symmetry index, which was calculated from the ratio between the sizes of the largest and the smallest chromosomes (Stebbins, 1971). Terminology of chromosome morphology based on the position of a centromere followed Levan (1964). For comparison among different karyotypes at mitotic metaphase, a karyotype formula was used.

2.3 Giemsa C-banding

The root tips were pretreated with 0.002 mol/L 8-hydroxyquinoline solution for 2 h at 18—20 ℃ and then fixed as above. They were rinsed in distilled water and then hydrolysed in 0.2 mol/L HCl at 60 ℃ for 8—10 min, and digested with 2% cellulase enzyme for 30 min at 37 ℃. They were subsequently squashed in the 45% acetic acid. The cover slips were removed by freezing in the liquid nitrogen and put in the pure ethanol for 1h, then dried in the air for 2days. The slides were incubated for 7 minutes in the saturated Ba $(OH)_2$ at room temperature and washed them quickly with distilled water. Then the slides were incubated in 2×SSC at 60 ℃ for 1h and be stained with 2% Giemsa (diluted in distilled water) for about 60—90 min.

Select the photos whose chromosomes are integrate and bands are clear to analyse the chromosomal bands. Refert the Karyotyping and further confirm them by Scanner and Microsoft photo editor, then synthesize the C-banding pattern.

3 Result

3.1 Karyotype analysis of *A. sinensis*

Somatic chromosome numbers for *A. sinensis* in three populations were all 2n=16(Plate I:1,2)and the basic chromosome number were x=8.

For the three populations specimens examined, the karyotype formula, chromosome length, genome length and asymmetry indexes are listed in Table 1. The karyotypical formula of three population was 2n=16=6m+6sm+4st) and they have same karyotype characters. They comprised 6 metacentric chromosome pairs and 6 submetacentric chromosome pairs and 4 subterminal chromosome pairs.

Even though the species studied were found to be homogeneous in karyotype formula, intra species variation related to chromosome size and genome length were observed. Population P3 had the biggest chromosome length range (2.76—6.96 μm), P1 an intermediate

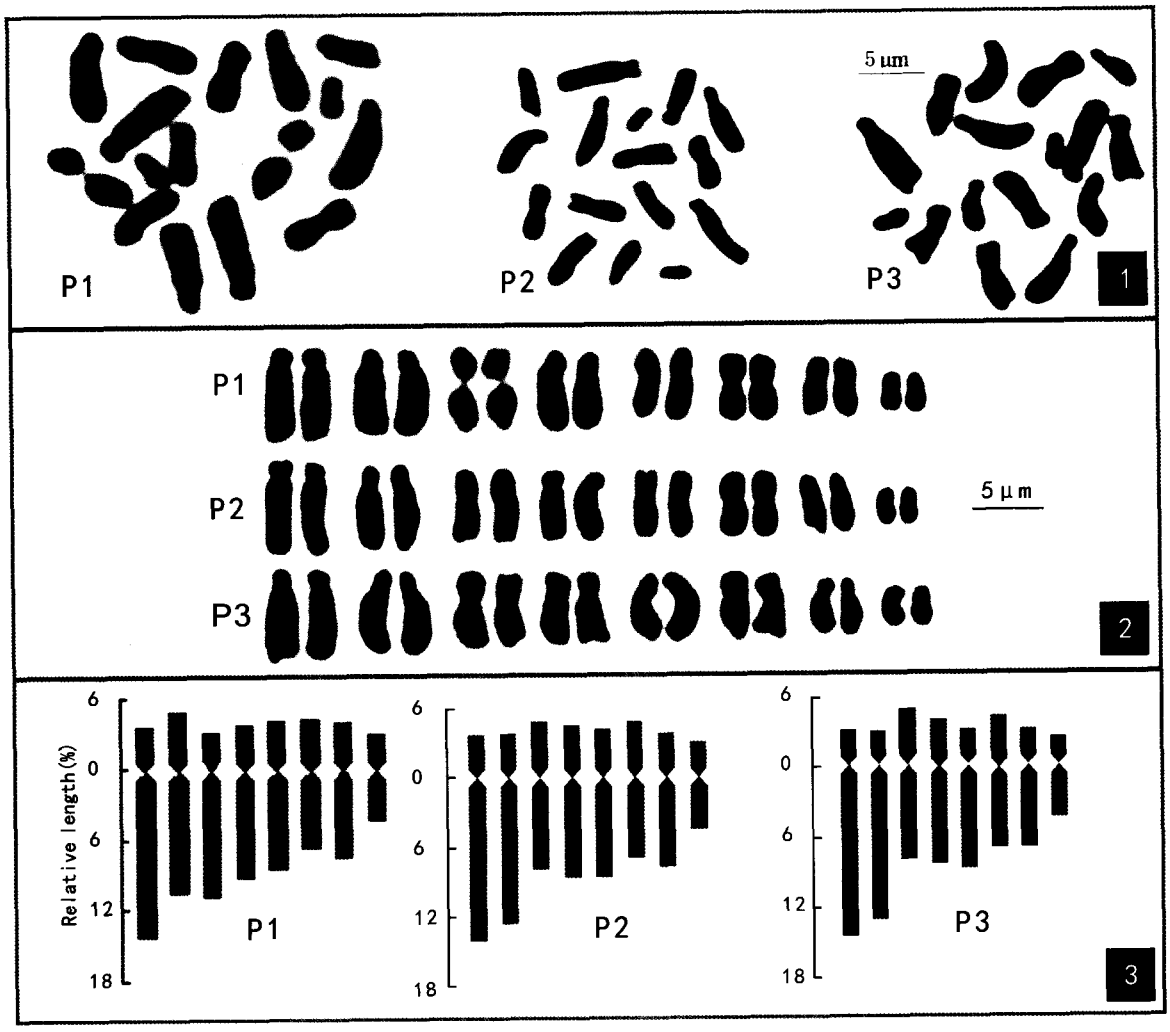

Plate I 1. Micrographs of chromosomes of *A. sinensis* (×1 000), 2n=16, Scale bar=5 μm; **2.** Chromosomes karyotype of *A. sinensis*; (Scale bar=5 μm); **3.** Idiograms of somatic metaphase chromosomes of *A. sinensis*.

size (2.87—6.96 μm) and P2 had the smallest (2.97—7.01 μm). Intergeneric variation in genome length was also observed. Mean genome length was 41.02 μm for P1, 41.83 μm for P2 and 40.08 μm for P3.

3.2 Gimesa C-banding of *A. sinensis*

Five pairs chromosomes have centromeric C-bands of population P1(Plate Ⅱ). Chromosome 2 and 6 have telomere bands on both the short and the long arms. Chromosome 4 and 5 have telomere bands on the short arms and intercalary bands on the long arms. Chromosome 3 has a completely heterochromatic short arm. The Giemsa C-banding pattern of P1 is $2n=2x=16=4C+2T+6CI_+ +2TI_+ +2T^+I_+$.

For population P2(Plate Ⅱ), 5 pairs chromosomes have centromeric C-bands, like population P1. Chromosome 1 has three C-bands, including two telomere bands and one intercalary band on the long arm. Chromosome 3 and 7 have centromeric bands and intercalary bands on the long arms. Chromosome 4 has telomere bands on both the short and the long arm. The C-banding pattern is $2n=2x=16=4C+2T+2CT+2CI_+ +2CT^+I_+ +4T^+I_+$ Like P1 and P2, population P3 also have 5 pairs chromosome with centromeric C-band (Plate Ⅱ). Chromosome 1 and 2 have completely heterochromatic short arm and intercalary band on the long arms, moreover, chromosome has one telomere band. Chromosome 3 and 5 have intercalary bands on the long arms. Chromosome 4 has intercalary bands on both the short and the long arms. Chromosome 7 has telomere bands on both the short and the long arms. The Giemsa C-banding pattern of P3 is $2n=2x=16=4C+2I+2T+2CI_+ +2CI_+T+2CI_+T^+ +2I_+T^+$.

Table 1 Parameters of chromosomes in the populations of *A. sinensis*

Location	Chromosome number	Relative length (%)	Arm ration	Type of chromosome
P1 (Nanning)	1	13.47+3.5=16.97	3.85	st
	2	10.06+5.71=15.77	1.76	sm
	3	10.32+2.98=13.30	3.46	st
	4	8.61+3.92=12.53	2.19	sm
	5	8.10+4.01=12.11	2.02	sm
	6	6.39+5.03=11.42	1.27	m
	7	7.07+3.84=10.91	1.84	m
	8	4.09+2.90=6.99	1.41	m
P2 (Maoming)	1	13.46+3.51=16.97	3.83	st
	2	12.04+3.61=15.65	3.35	st
	3	7.44+5.69=13.13	1.31	m
	4	8.11+4.26=12.37	1.90	sm
	5	8.19+3.93=12.12	2.08	sm
	6	6.61+5.10=11.71	1.29	m
	7	7.36+3.59=10.95	2.05	sm
	8	4.26+2.84=7.10	1.5	m
P3 (Tunchang)	1	14.4+2.97=17.37	4.85	st
	2	13.0+2.79=15.79	4.65	st
	3	7.94+5.50=13.44	1.44	m
	4	8.37+4.28=12.65	1.96	sm
	5	8.64+3.40=12.04	2.53	sm
	6	6.72+5.06=11.78	1.33	m
	7	6.71+3.32=10.03	2.08	sm
	8	4.18+2.71=6.89	1.55	m

4 Discussion

Three populations of *A. sinensis* examined in this study have a consistent somatic chromosome number of 2n =16. The ratio of the longest to the shortest chromosome in all three populations exceeded 2.00, indicating that the species we studied have a relatively high interchromosomal asymmetry. The chromosome number of *A. sinensis* agreed with that reported by Debenath *et al.* (1995) for *Aquilaria agallocha* from Sipahijala forest, India. But some differences still lies in these two species. The karyotype of *A. agallocha* is symmetric with 5 metacentric pairs and 3 sub-metacentric pairs, one of which has secondary constriction. The chromosome type of *A. sinensis* in three populations belonged to category 2B which was not so symmetric as *A. agallocha*. In these populations, there are more sub-metacentric pairs than *A. agallocha* and no secondary constriction occurred. *A. sinensis* comprised 2 pairs subterminal chromosome which didn't exisit in *A. agallocha*. And the chromosome researches on other species of genus *Aquilaria* have not recorded.

According to the classification proposed by Stebbins, the karyotypes of the three populations can be inserted within category 2B (categories range from 1A (most symmetrical) to 4C), a more asymmetry karyotypype. Stebbins regarded that the foundational trend of the karyotype evolution is from symmetry to asymmetry, during the systematic evolution, the majority of ancient or primitive plants have more symmetric karyotype. Whereas, asymmetric karyotype is often seen in the plants which are derivative or in the advanced stage of evolution. So *A. sinensis* can be considered as advanced as for their karyotype.

Table 2 Comparison of karyotypes among populations in *A. sinensis*

Population	Karyotype formulae	Ration of chromosome length	Average arm ration	Type of chromosome	Asymmetry cofficient
P1	6m+6sm+4st	2.43	2.22	2B	68.12
P2	6m+6sm+4st	2.39	2.17	2B	66.71
P3	6m+6sm+4st	2.52	2.55	2B	70.16

The C-bands of *A. sinensis* in three populations all showed centromeric bands, telomere bands and intercalary bands distributing among various chromosomes. But the number and position of C-bands among populations were different. C-banding showed conspicuous bands in numerous chromosome pairs, located at centromeric positions, with some pairs also characterized by C-bands at the telomeric position on the short arms or heterochromatic short arms. Giemsa C-bands of *A. sinensis* had not only centromeric bands but also telomere bands and intercalary bands, furthermore, bands patter variety didn't happen only on the short arms. C-bands differentiation of *A. sinensis* chromosome occurred both short arms and long arms, which indicated *A. sinensis* had advanced evolution level. It was suggested that C-bands had polymorphism among populations, which can be used as a genetic index to analysis relations among genus *Aquilaria* with high reliability.

The chromosomal karyotype displays the characters of the species at the chromosome set level, the chromosomal C-banding shows the spread of the constitutive heterochromatin in the chromosome(Jellen &

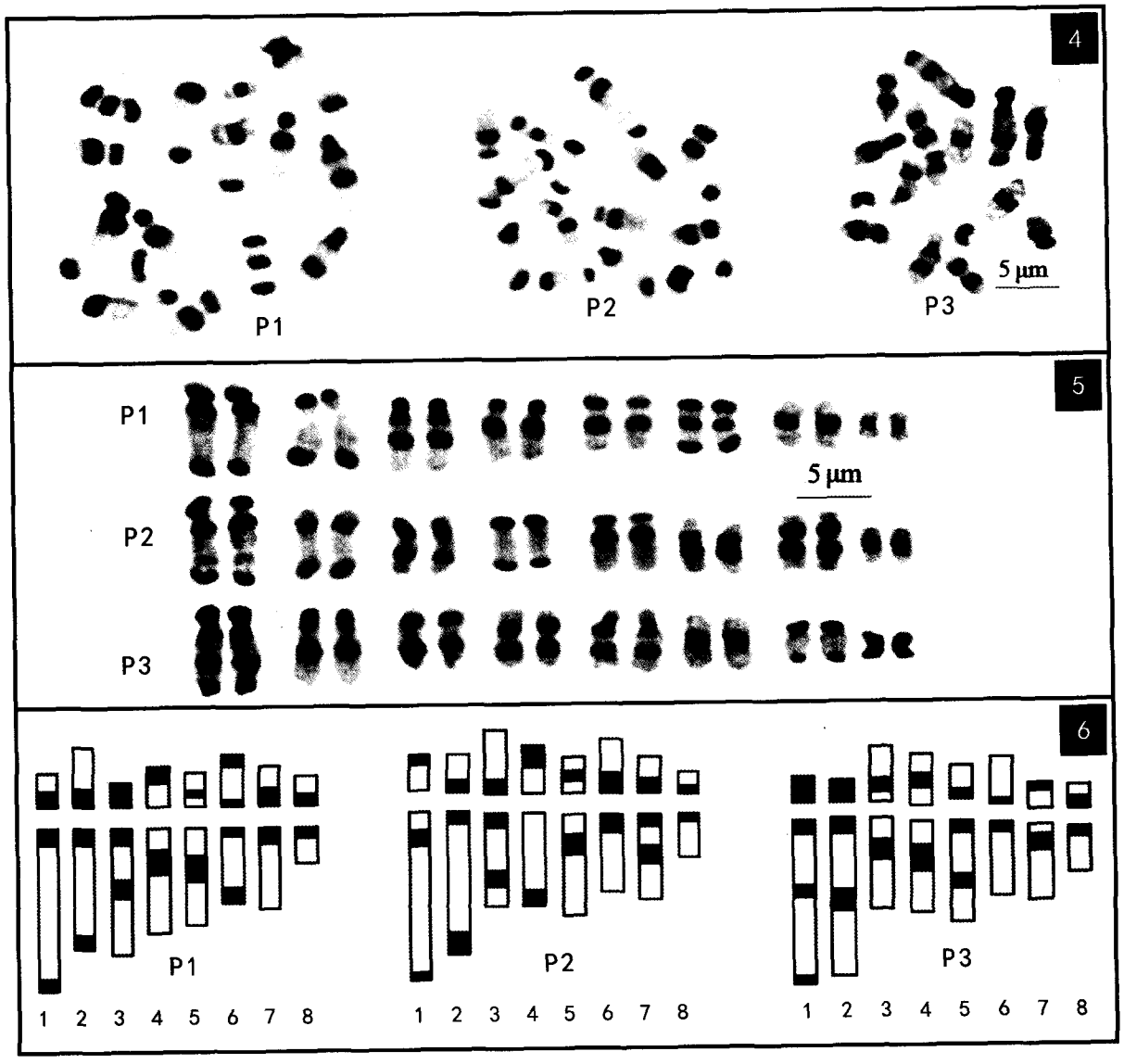

Plate Ⅱ **4.** Chromosomes C-banding of *A. sinensis* (Scale bar=5 μm); **5.** Idiograms of somatic metaphase chromosomes C-banding of *A. sinensis*.

Ladizinsky, 2000). The pattern of heterochromatin distribution has been determined for a large number of species and populations in studies that have investigated the possible polymorphisms and/or variations, in an attempt to understand phyletic interrelationships (Lourenco *et al.*, 1998). Because the polymorphisms of the chromosomal bands endows the specific recognition marker to the chromosomes and offer the evidences for genetic analysis among the different or the same species, so it can be used as a genetic marker to study the chromosomal operation species evolution and the relation of different species genome (Hüseyin & Sema, 2005).

The results of this study help to clarify the chromosomal differences among populations of *A. sinensis* with regard to the amount and location of heterochromatin. Once these banding patterns have been established, in other species a more detailed analysis of the evolutionary relationships of the species will be possible.

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本书是植物分类文献目录中最重要的工具书，是考证各类文献与时间（跨度从 1753 到 1940 年）的权威著作。**TL-2** 共 15 卷 11 318 页，包括 9 072 位作者的文献 37 600 条，编写时间前后达 32 年之久。无论是收集的内容还是覆盖的范围，该书都可以说是当今世界植物分类学界的宏伟巨作；不仅是对古典植物分类学文献，同时也包括当代的各种文献；不仅收录范围包括广义的植物学（藻类、真菌、地衣、苔藓、蕨类、种子植物），同时兼收古植物学以及少数生物学的内容。本书的编排方式以作者为序，按姓氏字母排列。每个作者都有详细的全名与缩写、生平、传记、工作单位、研究过的标本以及模式标本存放地点、出版物等介绍；同时还列举有关的参考文献以便读者进一步考证或获得更多的知识。每个作者的论著按年代排列，包括论著的全称与缩写、出版时间、地点、卷册，页码，图表，版次，以及相关的评论等。该书第一版仅一本，收录的论著 1 453 种，共 556 页。编者 Frans A. Stafleu(1921～1997)是荷兰人，不仅是 International Association for Plant Taxonomy (IAPT)的秘书而且长期工作于植物分类学界著名的 Utrecht University，是世界上著名植物学文献、历史和命名方面的权威；一人仅用三年的时间(1965～1967)完成这样的巨作，除了长期积累的资料外，更重要的是知识、智慧和阅历。第二版 Frans A. Stafleu 联合美国斯密森研究院国家自然历史博物馆(Natinal Museum of Natural History, Smithsonian Institution)刚卸任的主任 Richard S. Cowan(1921～1998)并扩充内容(参见 ***Taxon*** 28(1, 2/3), 77-86, 1979)，两人历时 12 年之久(1973 年 11 月到 1985 年 10 月)完成近 7 000 页的 7 卷本，其内容是第一版的 10 多倍，论著达 18 785 种；最终使该书成为世界植物分类学家必不可少的工具。然而由于第二版从第二卷即 1976 年开始得到美国国家自然科学基金(National Science Foundation)的资助，所以增加很多内容，但先行完成的第二版第一卷(即作者 A 到 G)是在没有经费的情况下进行的，和第二卷以后各卷相比需要补充。第二版完成后，Richard S. Cowan 由于已经退休而不参加余下的工作，于是 Frans A. Stafleu 又联合 Utrecht University 的 Erik A. Mennega(1923～1998)对第二版的第一卷进行补订。遗憾地是补订工作由于两位作者 1997 年和 1998 年先后故去，只完成 A 到 E(其中补编第Ⅴ和Ⅵ卷的内容 D-E 由德国柏林植物园的 Norbert Kilian 和 Ralf Hand 在前人的手稿基础上编辑完成)，尽管他们当初希望能够完成第一卷的全部 A 到 G。第二版第一卷的补编工作任务相当大，同时也增加很多内容，到补编Ⅵ时已经收载论著已达 33 658 种。幸亏美国斯密森研究院 Dan H. Nicolson 联合 Laurence J. Dorr 接过续编，完成补编余下的两册(F-Frer 和 Fres-G)并于最近出版。大多数中国植物学工作者都知道 Dan H. Nicolson 先生，不仅因为他是当代世界上植物分类学领域的著名权威人物，而且知识非常渊博；多次到过中国讲学，人更是非常友善，任何时候请教任何问题或是寻求帮助，从来都让你感到他不仅像一位学者，更是一位慈祥的长者。本书的作者们基于欧美主要具有历史性的植物学研究机构，收集的文献可谓植物分类学史上从未有过。然而本书仍有不足之处，正如作者在第二版第一卷前言中所指出的那样，遗憾地是由于语言等原因，东欧的斯拉夫语系地区及远东的工作收录的有限(当然包括中国)。该书的全部内容已经上网(http://tl2.idcpublishers.info/)，读者不仅可以检索，而且原始文献的单行本或者是缩微胶片的印刷品还可以付费索取。

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