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STUDIES ON THE IDENTIFICATION OF *Pleioblastus Simoni* IN KOREAN BAMBOOS

KWON, Oh Yong

(Dept. of Botany, Seoul National University)

權五溶 : *Pleioblastus Sinomi* Nakai (해장죽)의 同定의 關한 硏究

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ABSTRACT

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Studies on the anatomical observation of Bamboos distributed in East-South Asia for systematic botany were scarecely reported except for the morphological studies on Bamboos, which was investigated by Dr. Hiroshi Usui,[3)4)] present author[6)] and others. Recently it has been certain that it could be hardly classify some species of bamboo family without this work.

For this reason the author reported the anatomical studies on Korean bamboos in 1959, and furthermore the author intended to identify the two species of *Pleioblastus Simoni* Nakai in Korean bamboos by studying the characteristics of internal structure and qualitative assay of free amino acid containg in *Pleioblastus Simoni* Nakai (1), (2).

In view of fact that there is some diferences between two species, it is likely to be identifiable completely different species one another. Futhermore, a lot of characteristics surveyed by the author were as follow: It was reassumed that two species cultivated under same conditions for three years were different alternately in the species of *Pleioblastus Simoni* Nakai. At the same time, these studies could clarify some evolutional processes of bamboo family. On the other hand, it was found that two species had not only the obvious difference in aerenchyma, the size of thick-walled parenchyma and bulliform cell, but also the internode of *Pleioblastus Simoni* Nakai(1) is longer than the other, the speed of growing is more rapid, the leaf of former is narrow and that of latter is wide. The free amino acids containg in each species were found as 18 kinds, especially *Pleioblastus Simoni* Nakai (2) had not Histidine in spite of containg in *Pleioblastus Simoni* Nakai (1).

From the characteristics and the experiments described above, it seemed to the author that *Pleioblasus Simoni* Nakai (2), which was growing at the region of Pohang in Korea, was a variety of *Pleioblastus Simoni* Nakai (1) which was growing at the region of Ulsan in Korea.

INTRODUCTION

It has been recently acquainted for us that many kinds of Bamboos were distributed over Korea, China, Vietnam, Japan as the main particular product of East-South Asia. They are well grown in all the region of Kyungsang-province, Chunla-province which are belonging to the temperate zone of South

Korea. Owing to the development of handicraft in this country, the artificial cultivation of Bamboos is more increased than before. Few the literature on the study of Bamboo is published as yet with the exception of studies on *Sasa nipponica* and *Pleioblastus Chino*[3)4)] of Dr. Usui, and there is little reliable information available at the present time concerning the anatomical study on Bamboos.

For this reason the author reported on the anatomical study of Korean Bamboos at the convention of the Botanical Society in 1958 throughout the working for one year. Having collected 9 species of 4 genus in *Bambusaceae* of Korea, the study was compared with the main vein, side vein, size of vascular bundle sheath, thick-walled parenchyma, bulliform cell and intercellular spaces. At that time there were some questions derived form the anatomical comparison between *Pleioblastus Simoni* Nakai (1) and *Pleioblastus Simoni* Nakai (2).

In order to investigate aforecited some questions, I have been studied on the anatomical identification between two species and on the qualitative assay of free amino acid by paper chromatography from 1959 to 1962, and then the author is now willing to describe the result of experiments.

MATERIALS AND METHODS

The two materials of uninvestigated problems were *Pleioblastus Simoni* Nakai (1), which was growing at the region of Ulsan, *Pleioblastus Simoni* Nakai (2), which was growing at the region of Pohang. These two species were transplanted at the same place of Youngchun area on April 4, 1959 and were cultivated for three years under the same conditions.

Anatomical method. Having manipulated by Johansen Paraffin method, these materials the author observed the middle part, tip part, petiole part of the mainvein under a microscope and found out the value of average in the anatomical size. Every preparation were cut with the thickness of 10μ and stained with double stain, Safranin and Fast green.

Before cutting the materials, it was so hard that was softened by hydroflouric acid. According to above methods the author made one hundred preparation every materials, correctly compared with one another the vascular bundle, size of vessel, thick-walled parenchyma, vascular bundle sheath and bulliform cell by the micrometer.

Qualitative assay of Amino Acid. The preparation of sample for paper chromatography was manipulated in the following method; The amino acids of material which was growing some part of meristem situated on the tip stem of Bamboo were extracted by the common method of Moore & Stein, and the Ion Exchange resin was used before concentration. Theyare an extracted method and developed method of amino acids in the detailed processes which are carried out this experiments.

First, 3g. of dry material powder was taken as a sample and was extracted 3 times to 80% ethanol ···Sample was added with 40ml. ethanol for 24hrs. Dreg after the first extraction was added with 30ml. ethanol for 12 hrs. and dreg after the second extraction was added with 30ml. ethanol for 12 hrs. ···and the dregs were excluded every time by centrifugation (5000/times/min.) for 5 minutes. The extracted solution was mixed well with three times of chloroform, and the mixture was put aside until the separtating layer is clear, and then the upper layer was used for Ion Exchange. The liquid filtered from this column was concentrated at lower pressure and 40°C, until the sample solution becomes 1.5ml.

Secondarily, the development was proceeded two dimensional with Toyo paper 50 (25cm×33cm), in the constant temperature chamber of 18—20°C, and the sample spotting on this paper was taken 0.006ml. as a standard. The solvent used was water-saturated phenol (1:1) for one dimension, and butanol acetic acid solution (Butanol: Acetic acid: Dist. Water 4:1:5) for two dimension. Being finished the development, the paper was dried at 30—40°C and sprayed with 0.2% ninhydrin solution. The Rf. value of the spot which appeared at 80—90°C was compared with that of each standard

amino acid and the Rf. value found with several reports are added to these results.

EXPERIMENTAL RESULTS

Anatomical characteristics.

The structure vascular bundle: There are the collenchyma of 20—23μ under both epidermis, many sclerenchymatous cell inside of crossed leaves and some vascular bundle near the middle part of crossed leaves. Generally the thick-walled parenchyma and collenchyma of petiole is thickened more than the other tip and middle part, and the thick-walled parenchyma is well differentiated. Especially the structure which is situated in these two species of *Pleioblastus* genus is different as follow:

Table 1.

Species	diameter
Pleioblastus Simoni Nakai (1)	120—140μ
Pleioblastus Simoni Nakai (2)	95—100μ

Thus many differences in the structure of vascular bundle appeared as Table 1 despite of the same species. So it is assumed that these two species are different alternately in view of the anatomical observation.

Vascular bundle sheath: This is similar to the circle form, enclosed with xylem and phloem, and it is situated under the middle part of main vein. There is not nearly different between vascular bundles of two species. they have about the thickness of 10—20μ and it is composed of many small cells as the circle.

Vessel and Sieve tube: Sieve tube generally consist of small sieve cells like the half-circle form and it is apparently differed from vessel because of vascular bundle sheath situated outside. Three vessel which are situated at the upper part are the pitted vessel or ring vessel, and the size of structure is as follow:

Table 2.

Species	diameter
Pleioblastus Simoni Nakai (1)	20—22μ
Pleioblastus Simoni Nakai (2)	20—21μ

In Table 2 it is not many differences between two species of them.

Thick-walled parenchyma: Thick-walled parenchyma which consist of many sclerenchymatous cells form the most part of mesophill in the main vein of leaf. Thick-walled parenchyma around vascular bundle is small cell, but it is gradually crowded with large sclerenchymatous cell. The difference of thick-walled parenchyma thickness between two species is as below:

Table 3.

Species	thickness
Pleioblastus Simoni Nakai (1)	70—80μ
Pleioblastus Simoni Nakai (2)	60—70μ

The difference in this table is 10μ.

Side vein: Bamboos which belong to monocotyledon have a parallel venation and they are connected with the anastomosis. Side vein means so a parallel venation of leaf that it is situated on a certain distances. There is side vein at both side of main vein, the situation of side vein settled according to the outside structure of each species and there is no thick-walled parenchyma around the vascular bundle of side vein such as main vein.

Table 4.

Species	diameter
Pleioblastus Simoni Nakai (1)	76—92μ
Pleioblastus Simoni Nakai (2)	85—93μ

Side vein that is smaller than the vascular bundle of main vein has a little difference between *Pleioblastus Simoni* Nakai (1) and *Pleioblastus Simoni* Nakai (2).

Other Characteristics

The difference of growth between two species: The growth of *Pleioblastus Simoni* Nakai (1) is earlier than the other in the same condition of culture environment. At the same time the reproduction rate of the former was more active than those of the latter as much as anybody obviously can distinguish them.

Table 5.

Species	1*	2*	3*
Pleioblastus Simoni Nakai (1)	10—15cm	20—23cm	50—60cm
Pleioblastus Simoni Nakai (2)	8—12cm	13—15cm	23—35cm

* 1: The height of stem grown for one month
2: 〃 for two month
3: 〃 for three month

The inside structure of leef: There are many stomata, bulliform cell in the upper epidermis and many long cells under the upper epidermis. Aerenchyma is greatly formed into the inside of leaf according to the difference of each species, there are also many stomata in the under epidermis of leaf and the elliptic spongy parenchyma on the upper part of leaves.

Small vascular bundle is situated near the upper epidermis at the base of spongy parenchyma from the main vein to all the side vein. Particularly the aerenchyma of *Pleioblastus Simoni* Nakai (1) is much developed than the other because of having large intercellular space. It is presumed that we can identify the difference between two species in this point of view.

The arrangement of vascular bundle: There is a large vascular bundle in the inside of main vein and are many small vascular bundle in a ring form around large vascular bundle. Small vascular bundles which are arranged in a ring form on the sectioned middle part of main vein were smaller than the vascular bundle of petiole. Some anastomosis are situated between the main vein and the side vein and some small vascular bundle are arranged in a streight line on the upper part of spongy parenchyma. Thus a number of small vascular bundle in anastomosis is different by a kind of leaf.

Assimilation tissue: There is thick walled parenchyma so called assimilation tissue between the upper epidermis and the under epidermis. A lot of chlorophyll are contained in each cell of assimilation tissue and a number of cell in this assimilation tissue are adoptly differentiated to carry much assimilation matters. The palisade parenchyma of leaf consist of two cell layer and it is bordered with spongy parenchyma near the under part of leaf, but we can hardly find a obvious difference between two species.

Bulliform cell: It is much developed in *Gramineae* as the characteristics of them and it has not any chlorophyll, contains much tannin in the cytoplasm with non-color. The difference shown in a size of bulliform cell between two species is obviously found.

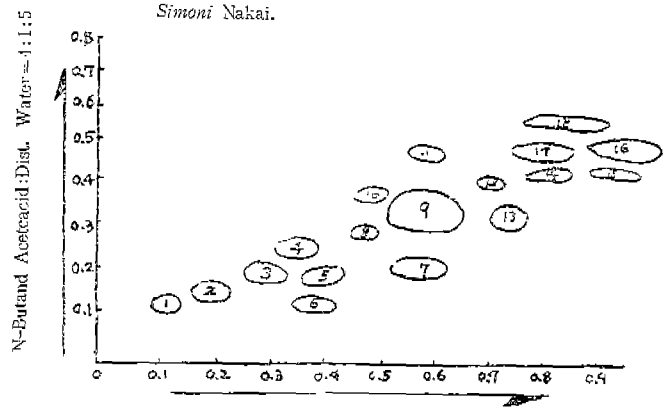
Table 6.

Species	thickness
Pleioblastus Simoni Nakai (1)	70—80μ
Pleioblastus Simoni Nakai (2)	40—65μ

Intercellular space: There are many intercellular space between the palisade parenchyma and the spongy parenchyma, and it is helped the aerenchyma by adopted differentiation. Especially the high differentiation of aerenchyma means that the intercellular space is much developed. This intercellular space seems to be a cell, but it has not any so chlorophyll that it is distinguished from the assimilation tissue. Many difference between two species is apparently appeared in this intercellular space and parenchyma; *Pleioblastus Simoni* Nakai

(1) has large intercellular space, but on the contrary *Pleioblastus Simoni* Nakai (2) has smaller intercellular space than the former.

Fig. 1. Qualitative Assay of Amino Acid contained in *Pleioblastus Simoni* Nakai.



1. Aspartic acid	2. Glutamic acid	3. Serine
4. Glycine	5. Arginine	6. Asparagine
7. Glutamine	8. Threonine	9. Alanine
10. Unknown A	11. Tylosine	12. β-Alanine
13. Histidine	14. Tryptophaine	15. Methionine
16. Penylalanine	12. Valine	18. Leucine-Iso Leucine

The free amino acids contained in *Pleioblastus*, 2 species were found as 18 kinds, but one of them was not to be identified yet. The qualitative content of free amino acid contained in *Pleioblastus Simoni* Nakal (1) was similar to that of *Pleioblastua Simoni* Nakai (2), when Rf. value of free amino acid was measured on the paper with chromatogram. The correct differences between two kinds, however, was indicated that the species of *Pleioblastus Simoni* Nakai (2) did not contain Histidine, while it them in the species of *Pleioblsstus Simoni* Nakai (1).

The distribution of free amino acid in each species is shown in Table 7.

DISCUSSION AND CONCLUSION

Nowadays a few of people have scarcely begun the study on the external features of Korean Bamboos and some studies on the extrernal features of them have been only acquainted for the author. However it would be likely that the research direction due to scientific systematics attempts not only in the external features of plants, but also has to identify each species at the base of obvious anatomical atudy and physiological diversity. From the biochemical aspects as the other aspect, if it is true that the characteristics of the species and its sub-division are naturally inserted into the physiology of the organism and its relation with its ecological niche, it is to be expected that the biochemical traits which are transmitted from organism to organism. The utility of superspecific categories is above all to enable us to express our views on the probable nature of physiology. As Calman(1949) said, "the characters most important in taxonomy are those which maintain themselves unchanged through het greatest of range of variation". Having considered this important point, it seems to the author that the system of classification in bamboo family could be studied.

First, many differences between the two species have especially appeared in the structure of vascular bundle of mainvein, intercellular species; the difference in the structure of vascular bundle of main vein is about 43μ between the two ispeces and it appaered many difference as Table 1.

Secondarily, the two species were comaered with

Table 7.

Material / Amino acid	*Pleioblastus Simoni* Nakai (1)	*Pleioblastus Simoni* Nakai (2)
Aspartic acid	+	+
Glutamic acid	+	+
Serine	+	+
Glycine	+	+
Arginine	+	+
Asparagine	+	+
Glutamine	+	+
Alanine	+	+
Tylosine	+	+
Threonine	+	+
β-Alanine	+	+
Histidine	+	−
Tryptophane	+	+
Methionine	+	+
Penylalanine	+	+
Valine	+	+
Leucine-Isoleucine	+	+
Unknown A	+	+
Total	18	17

a little difference in the structure of vessel, the differences of 10μ in the thickness of thick-walled parenchyma, the difference of about 26μ in bulliform cell and the difference of about 10μ in the side vein.

Thirdly, there are some difference in the external structure of the two species; the leaf of *Pleioblastus Simoni* Nakai (1) was more narrow than the other and in the length of stem *P. Simoni* Nakai (1) was longer than the species of *P. Simoni* Nakai (2).

Finally, there are some differences in the qualitative assay of free amino acid between two species which is shown in Fig. 1.

Synthesizing above all statements synoptically it must be sure that the two species are quite different to each other and it is, therefore, assumed that *Pleioblastus Simoni* Nakai (2) is proper to be placed in a taxonomy of *Pleioblastus Simoni* Nakai(1).

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摘 要

本試驗은 3年間 同一한 條件下에서 生育한 해장죽(1)과 해장죽(2)의 比較解剖學的 硏究를 基盤으로 하여 서로 相異한 種임을 再確認하였다.

組織學的 硏究에 依하여 대체의 進化過程을 多少 究明할 수 있었다. 主脈維管束의 크기, 通氣組織, 導管의 크기, 厚壁柔組織의 肥厚, 踏型細胞에 있어서 두種의 뚜렷한 差異를 發見하였다. 外部形態에 있어서도 해장죽(1)과 해장죽(2)는 相違하였으며 해장죽(1)은 節間이 길고 해장죽(2)보다 狹葉이며 同一한 環境條件에 있어서 生長率의 差가 生겼다. 두種間의 Paper chrmatography에 依한 定性分析에서도 相異하였다.

以上 諸要因에 依해서 해장죽(1)과 해장죽(2)는 相違한 種으로써 해장죽(2)는 해장죽(1)의 變種임을 結論지을 수 있었다.

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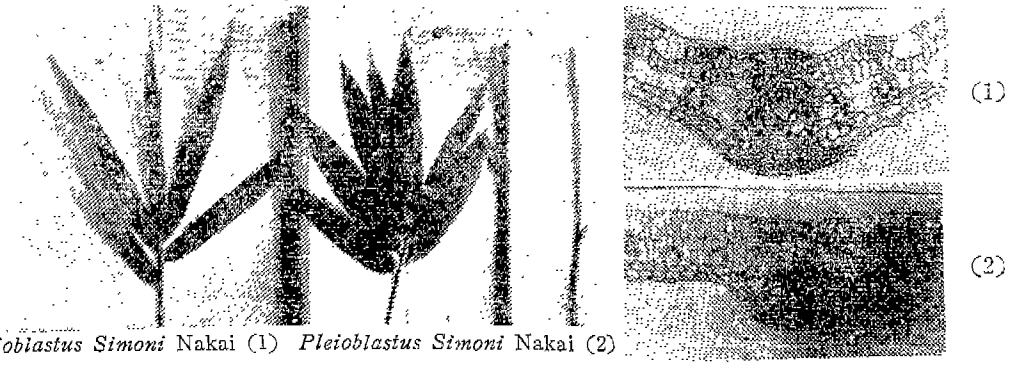

Pleioblastus Simoni Nakai (1) *Pleioblastus Simoni* Nakai (2)