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Microstructural Properties of Tropical Legume Seeds

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열대지방에서 재배되는 종실의 미세구조에 관한 연구

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Abstract

Microstructures of 18 kinds of legume seeds (15 varieties including 3 strains) which were cultivated in tropical areas, were observed under a light microscope. Majority of legume seeds were composed of starchy cotyledonary cells in which large amounts of single starch granules were contained, while a few had cotyledonary cells filled with a number of protain bodies. Starch granules were different in size and shape depending on varieties. Some contained lipid bodies distributed in cytoplasmic network, and were distinctive in thick cell walls. Microstructure of soybean was also observed for the comparison of the structures.

Introduction

The family leguminosae includes numerous varieties of species and are widely distributed from the tropics to the arctic regions. Some number of them have been cultivated for foods and feeds since remote antiquity. Especially in south Asian countries, several seed legumes have been traditionally used as staple food and taken a role of protein supplement in the habitants' diet, since the percentage of protein in cereal grains is 10-15% of dry matter and legume seeds 20-40%

In this paper,microstructural properties of some varieties of tropical legume seeds were observed mainly under a light microscope, some under a transmission electron microscope.

Materials and Methods

Materials

Tropical-grown legume seeds were obtained from Tropical Agricultural Research Center of National Food Research Institute. Soybeans were Japanese domestic products. The kinds of legume seeds and countries produced are as follows:

Benas (*Phaseolus Vulgaris*, India), Black gram (*Vigna mungo*, India), Chick pea (*Cicer arretinum*, India), Cow pea (*Vigna sinensis*, VS 56-4 and VS 58-5, Indonesia), Cluster bean (*Cyamopsis tetragonolaba*, India), Green gram (*Vigna radiata*, India and *Vigna radiatus* PR-67, Indonesia), Hyacinth bean (*Dolichos lablab* DL 54-3 and DL-10, Indonesia), Khesari (*Lathyrus, Sativa*, India), Lentil((*Lens esculenta*, India), pea (*Pisum sativum*, India), Pigeon pea (*Cajanus cajun*, India), Rice bean (*Vigna calcartus*, Thailand), Sword bean (*Canavalia ensiformis*, Indonesia), Winged bean (*Psophocarpustertragonolobus*, Thailand), Yam bean (*Pachyrrhizus erosus*, Thailand), Soybean (*Glycinmax*, Japan).

Preparation of microscopic specimen

Small pieces of cotyledonary tissue in each legume seeds were cut out with a razor blade, fixed with 5% glutaraldehyde solution and then with 1% osmium tetroxide solution (both in phosphate buffer containing 2.5% sucrose, pH 6.7), dehydrated with a graded acetone series (40% to 100%) and embedded in Epon resin. For a light microscope (LM), the blocks prepared as described above were sliced to about 10μm thickness with an LKB ultratome and affixed on a glass slide. Affixed specimen were stained with 0.5% solution of Coomassie Brilliant Blue in 7% acetic acid-50% methanol overnight and decolored with 7% acetic-50% methanol. Polysaccharides were stained with Schiff's reagent after oxidation with 0.5% periodic acid solution. Lipid were stained with a saturated solution of Sudan Black B in 50% ethanol (1). For a transmission electron microscope (TEM, JEM EX-1200) the blocks used for the light microscope were ultra-thin sliced and the specimens were double-stained with saturated uranyl acetate solution, and then saturated lead acetate sollution which was filtered just before staining (1).

Results and Discussion

Legume seeds used in the present experiment are classified into Vicieae and Phaseoleae in Papilionaceae as follows: Viciae; vicia, cajanus, cicer, lathyrus, lens, pisum. Phaseoleae; dolicholos, glycine, pachyrrhizus, phaseolus, psophocarpus, vigna. It is uncertain where cluster bean (cyamopsis) belongs to.

Microstructues of Starch-rich Legumes

Fig. 1 shows light micrographs of cotyledonary tissue in cow pea (vigna, VS 56-4) stained by the three methods; Coomassie Brilliant Blue (CCB), periodic acetic acid-Schiff (PAS) and Sudan Black B (SB). In any case, starchy cells of cow pea could be clearly observed, in which single starch granules ranged 10-30 μm in diameter were distributed. Starch granules and cell wall were stained with PAS, but were not stained with CBB and SB. Cytoplasmic matrix between starch grains was stained faintly with SB. Intracellular spaces between each cell were observed by the three methods.

As the principal feature of legume seed structure could be ovserved by any three methods, one of the most clear photographs was selected for each legume seed for Fig. 2 shows light micrographs of cotyledonary tissue in 16 knids of legume seeds (either strains of cow pea, green gram or rice bean showed almost similar structures) used in the present experiments. Green gram, black bean, cow pea which belong to vigna species show similar structure, having a number of round or ellipsoidal or kidney-shaped starch granules ranged 7-35 μm in diameter, and benas (phaseolus) and hyacinth bean (dolichos) were also similar. However rice bean was different having bigger ellipsoidal starch granules with irregular swelling, in spite of being in vigna species. The starch granules were rather resemble to the ones in pigion pea (cajanus) ranged 15-70μm in diameter and located a few in a cell. Chick pea (cicer) seemed to be poorer in size and number of starch granules. The structure of starch granules in pea (pisum), sword bean (canavalia), lentil (lens), khesari (lathyrus) were not worth special mentioning but their cell walls looked thicker than others. In all kinds of legume seeds described above, the main cellular material was single (rarely semi-aggregated) starch granules, not compound or aggregated starch granules like rice, oat etc.

Microstructures of Protein-rich Legumes

The remaining four kinds of legume seeds (including soybean) showed quite different cellular structures. Yam bean (pachyrrhizus) and cluster bean (cyamopsis) had a number of globular materials which seemed to be protein bodies, and winged bean and soybean certainly contained

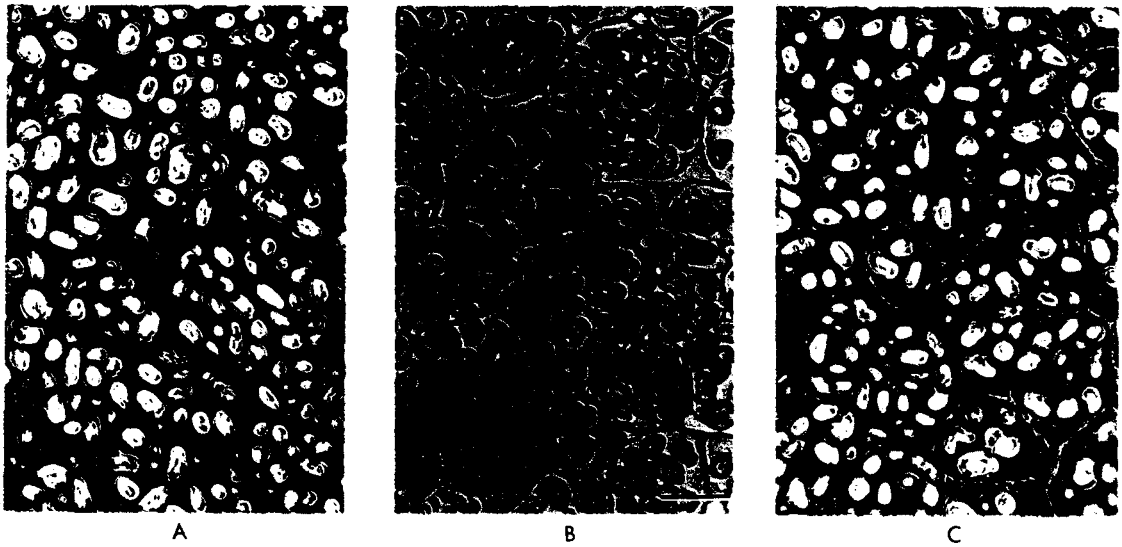

Fig. 1. Light micrographs of cow pea(Vigna sinensis, VS 56-4) stained three methods.

A: Coomassie Brilliant Blue (CBB)
B: Periodic acid-Schiff (PAS)
C: Sudan Black B (SB)
Direct magnification; x100, bar shows 50 μm. S:starch granule

many protein bodies, but all of them had none or a trace of starch granules. The globular materials in yam bean and cluster bean were stained with PAS and faintly stained with CBB. The seeds of yam bean has seldom been utilized, although the freshy turnip-like root of yam bean are used as food in tropical countries, central America and China. On the other hand, cluster beans (Guar beans) are resource of glucono-mannan (Guar gum) and also contain protein in cotyledonary cell. A little were reported on its protein (2,3).

Plasmolysis in Some Legume Seeds

Fig. 3 shows light micrographs of green gram (PR 67, Indonesia) and hyacinth bean stained with PAS in higher magnification. In green gram, cell wall was very thick with pit-pairs (showed by arrows) and starch granules were irregularlly swollen, while in hyacinth bean which looked like having thick cell walls, plasmolysis slightly occured and starch granules regurally swelled. In figure 3, the other sub-cellular materials located between starch granules were slightly observed in higher magnification. Compared with the micrographs of hyacinth bean in figre 3, plasmolysis of khesari and pea (figures 2-H and J) seemed to be also happened after the preparation of micrographs.

TEM Images of Legume Seeds

Fig. 4 shows transmission electron micrographs of cow pea and winged bean. Protein bodies in cow pea were 1-3 μm and faint in electron density while the ones in winged bean 2-5 μm and dense. In winged bean, a large number of lipid bodies were occupied in matrix between protein bodies, starch granules were rarely found and the thick cell wall with pit-pairs was observed. Such structure which are

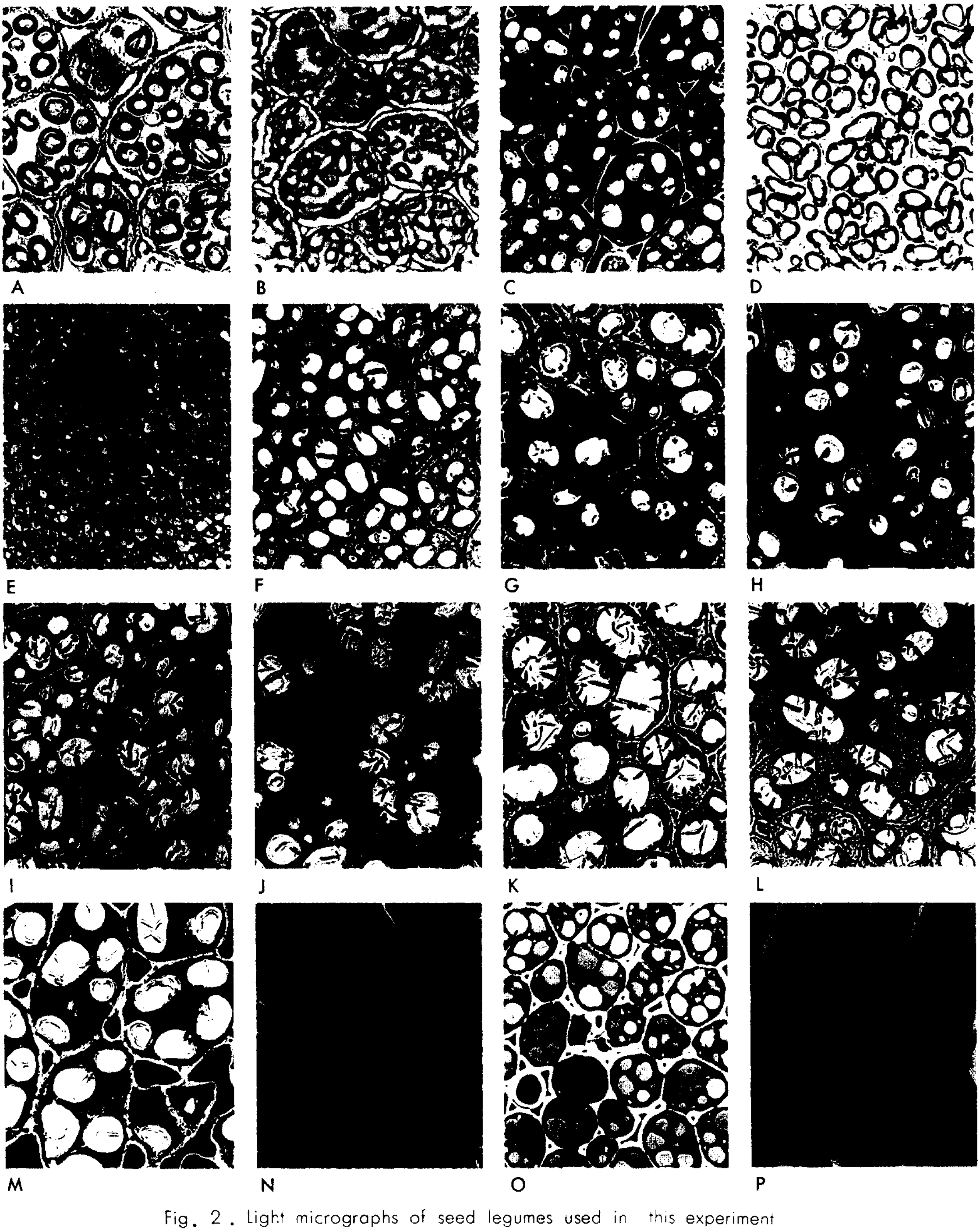

Fig. 2 . Light micrographs of seed legumes used in this experiment

A: Benas (PAS) B: Black gram (PAS) C: Chick pea (SB)
D: Cow pea (VS 58-5) (PAS) E: Cluster bean (SB)
F: Green gram (radiata, India) (CBB)
G: Hyacinth bean (DL 54-3) (SB)
H: Khesari (CBB) I: Lentil (CBB) J: Pea (CBB)
K: Pigeon pea (SB) L: Rice (CBB) M: Sword bean (SB)
N: Winged bean (PAS) O: Yam bean (SB) P: Soybean (CBB)
Direct magnification ; x100, bar shows 50 μm

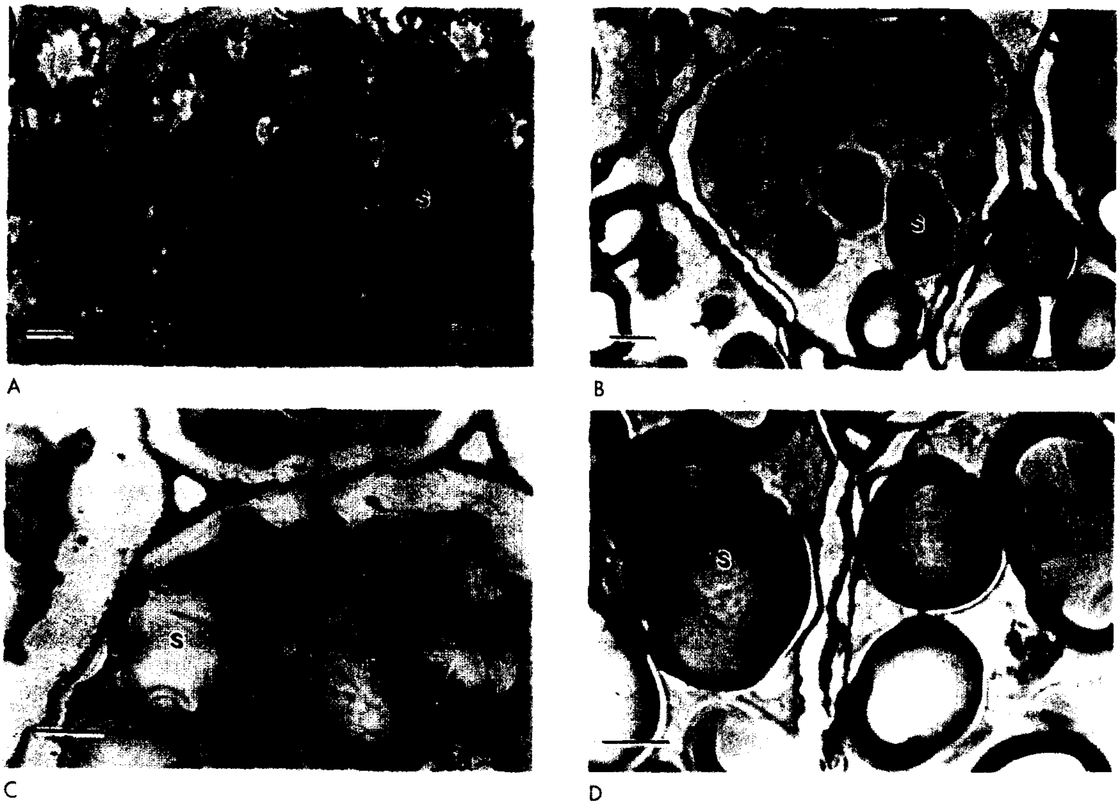

Fig. 3. Light micrographs of green gram and hyacinth bean in high magnification

A: Green gram (X300) B: Green gram (x500)
C: Hyacinth bean (x300) D: Hyacinth bean (x500)
Each bar shows 10 μm S: starch granule, arrow; pit-pair

rich in protein and lipid bodies is similar to that of soybean, but the cell wall of soybean is much thinner [4].

Winton and Winton [5] reported the microstructure of legume seeds and they showed dimensions of starch granules in maximum diameter on a table. According to their data, maximum diameter of starch granules varied from 27 to 80 μm. Rough hairy vetch (*Vicia hirsuta*), moth bean (*Phaseolus aconitifolius*), tepary (*Phaseolus acutifolius* var. latifolius) etc. were small and rice bean (*Vigna calcaratus*), chickling vetch(*Lathyrus sativus*), adzuki bean (*Phaseolus angularis*) etc. were comparatively large. Our results also showed that rice bean had big starch granules. It might be suggested that rice bean,pigeon pea and other legume seeds which have big starch granules may be used as good substitutes for adzuki bean in ann-making, since unbroken starch granules in ann paste prepared by the traditional processing make the characteristic texture of ann for confectionary use (6).

Winged bean has been calling an attention as a potential protein resources, however the seeds of yam bean have not noticed. In the case of winged bean,it was reported that thick cell walls prevented the easy milling and soaking in water but was digested by fermentation (7). It is interesting to note that the tuberous roots of the two have been used as foods. As described above,

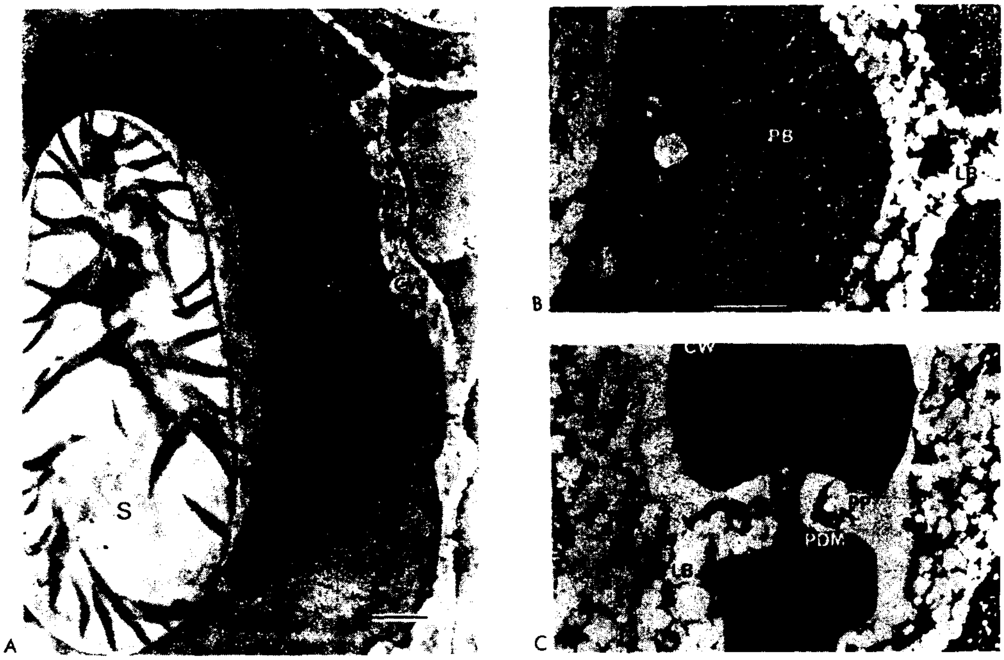

Fig. 4. Transmission electron micrographs of cow pea and winged bean.

A: Cow pea B: Winged bean C: Winged bean
Each bar shows 2 μm. S: starch granule, PB: protein body

CW: cell wall, PP: pit-pair, PDM: plasmodesmata (connection between cells, appeared at wall in pit-pair)

cluster bean has mainly used as resource of glucono-mannan but the residue has been reported to have 55% of protein.

In conclusion, such microstructural approach to legume seeds could suggest the possibilities for food uses in different angle from chemical and/or physical techniques.

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요 약

열대지방에서 재배되는 18종의 콩종자의 구조적인 특성을 분류하기 위하여 그 미세구조를 주로 광학현미경으로 조사하였다.

vicieae에 속하는 종실들은 많은 단일전분입자들로 구성된 자엽세포구조를 갖는 starch-rich legume이였으며, phaseoleae중에서는 benas(phaseolus), cow pea, green gram(vigna), hyacinth bean(dolicholus)이 starch-rich legume이였다.

한편 soybean(glycine), winged bean(psophocarpus)는 자엽세포가 대부분 protein body로 구성된 protein-rich legume이고 yam bean(pachyrrhizus)와 cluster bean(cyamopsis)에서는 protein body 보이는 구형물질로 이루어진 자엽세포 구조를 볼 수 있었다. 또한 green gram과 winged bean은

soybean에 비하여 두꺼운 세포벽을 갖고 있었으며 pit-pair가 관찰되었다. Lipid body는 winged bean과 soybean에서 볼 수 있었다.

starch-rich legume들은 팥고물 제조과정에서 전분입자들이 파괴되지 않음으로써 특징적인 조직감을 부여하는 red bean이나 benas와 같은 phaseolus의 대체자원으로 제시될 수 있었다.

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