Morphogenesis of Endosperm Tissue in Rice

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The studies on the mechanisms of histological development and reserve substance accumulation of endosperm in rice are absolutely necessary not only for the analysis of ripening mechanism but also for the fundamental investigation on rice quality, especially on eating quality.

Many researches have been made on the development of rice kernel, attaching importance to embryogenesis from the standpoint of genetics or breeding.

The morphogenesis of endosperm has been elucidated to a considerable extent by the studies on early developmental process[1], differentiation of aleuron layer[1,14] and starch-cell tissue[13].

But more detailed histological studies are now needed according to the recent development of ripening physiology and quality research.

The outline of the author's recent study on the morphogenesis of endosperm in rice shall be described in detail as far as possible[2-12].

Division and multiplication of endosperm cells

Fertilization is achieved in the evening on the day of anthesis. The formation of endosperm tissue in the embryosac starts from the evening of that day.

The first stage of endosperm development is the nuclear stage in which nuclear division is performed every six hours. The first peripheral layer, which consists of about 3,200 nuclei connected with each other in a line by protoplasm, is formed covering the interior surface of embryosac at the end of the third day.

On the fourth day, the secondary inner layer is accomplished as the result of simultaneous nuclear division. And immediately after, the formation of cell wall begins from the side of the embryonic pole around nuclei. After this stage, endosperm develops into cell stage and increases by cell division. Most of the cell divisions (86%) are carried out among the cells situated in the extreme outer layer of the endosperm and cells increase in the longitudinal, dorso-ventral and lateral directions of the rice grain. (Fig. 1)

A diurnal rhythm (daily cycle) exists in the cell division, that is, most of the cell divisions (50–72%) are achieved from midnight to early morning and cease during daytime.

Thus, at the end of the ninth day of cell division, it can be seen with the cross section of the middle portion of the rice grain that there exist about 16 cell layers from the central point to the ventral surface, about 20 cell layers to the dorsal surface and about 15 layers to the lateral surface (Table 1).

With the longitudinal section, about 150 cell layers exist in the dimensions from the embryonic end to the top end.

No more increase in the number of cells can be seen on the tenth day and afterward. Consequently, all the endosperm cells, determined on the ninth day, number about

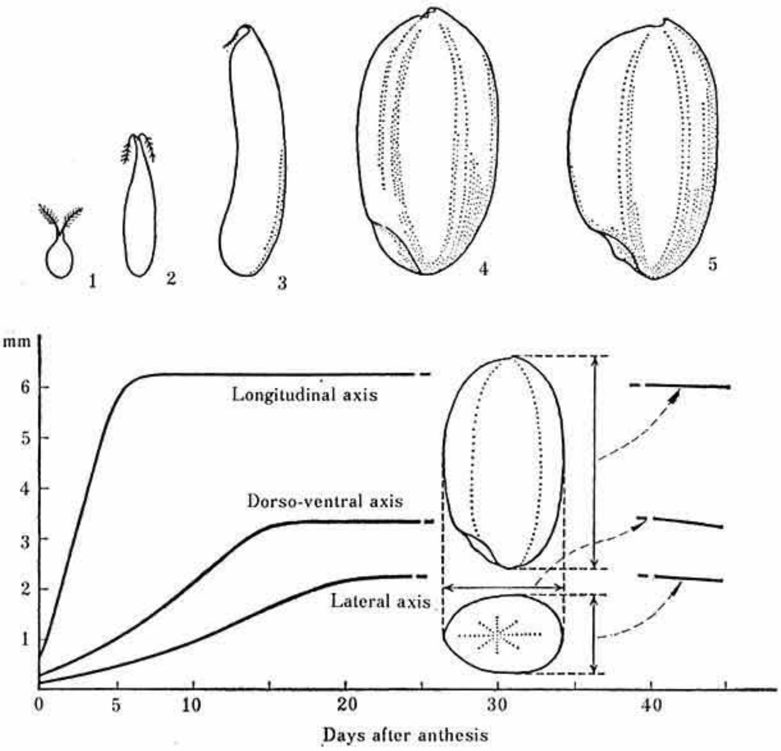

Fig. 1. Development of rice grain (Cultivar. Yoneshiro in normal season culture)
1—Day of anthesis
2—2nd day
3—6th day
4—25th day
5—40th day
(Hoshikawa 1972)

Table 1. Size of radii and number of cell layers in the cross section of middle part of endosperm tissue in different stages of development
(cultivears Yoneshiro)

Days after anthesis	Dorsal radius		Ventral radius		Lateral radius*	
	Length	Cell layers	Length	Cell layers	Length	Cell layers
	(μ)		(μ)		(μ)	
5	360	10.5	315	10.0	165	6.5
7	975	15.4	765	14.9	450	11.7
10	1560	19.8	1215	16.4	450	11.7
12	1650	19.9	1095	16.0	728	16.4
15	1875	19.5	1170	15.0	853	14.4
25	1785	19.5	1065	14.5	863	13.3
35	1785	20.3	1170	16.3	945	14.2
45	1870	19.3	1950	16.0	900	13.4

* Mean length of radii from the "central point" to both of the lateral surfaces of the endosperm
(Hoshikawa 1967)

180,000. This number is common to about 60 varieties of rice cultivated in Japan. Generally the number of endosperm cells of upland rice is a little more than that of the lowland rice.

As the result of the investigation on the number of endosperm cells of the 82 varieties cultivated in foreign countries (Table 2), the varieties of China (Khu rice), Korea, Taiwan and California (American short-grain rice) have from 150,000 to 180,000 endosperm cells, while the varieties of India and Burma are a little less in number ranging from 80,000 to 120,000. Moreover, the African and Western Europe varieties (including American long-grain rice) have more endosperm cells—from 190,000 to more than 200,000.

The total number of endosperm cells is related to the number of cell layers in the

Table 2. Relationship between whole number of cells and size of endosperm in paddy rice varieties

Size class mm³	Number of cells (10^3) VS ~100.9	S 101.0~129.9	M 130.0~159.9	L 160.0~189.9	VL 190.0~
VS ~19.99	Ip, Ip, Ip, Ip	Cs, Ph			
S 20.00~26.99	Ip, Ip, Ip, Ip Cs, Ck, J, Ph	Ip, Ip, B, Cy, Cy Cy, Cs, J, Ph	B, B, B, E H, K, K, Ph	V, Ip, Cy, K Am, Al, Al	
M 27.00~33.99		Cs, Cs, Ph Cy, Cy, H	B, CsCk, Ck, Ck K, H, (J)S, E, Sp	Ck, V, J, S, I H, E, E, E, Sp As, Am, Al, Al	
L 34.00~40.99			J	S, S, As, I, F	S, J, J, B, Sp I, I, F, F
VL 41.00~				Ph	F, I

Note: Varietal names are replaced by regional names which the varieties are cultivated
Regional names are abbreviated as follows:
As: America, short grain, Am: America, middle, Al: America, long, B: Burma, Ck: China khu, Cs: China shen, Cy: Ceylon, E: Egypt, F: Africa and Madagascar, H: Formosa, Ponlai, I: Italy, Ip: India and Pakistan, J: Java and Sumatra, (J): Japan, mean, K: Korea, Ph: Philippines, S: Soviet, Sp: Spain, V: Viet Nam and Laos

radius of the grain.

As for the number of cell layers in the rice grain radius, Japonica type varieties are similar to the common Japanese varieties but Indica type varieties are from four to eight and from two to three less in dorso-central and centro-ventral radii respectively than the Japonica type.

Consequently, the number of cells in the ventral radius is practically the same as that of the dorsal radius or sometimes the former is more than the latter. This fact is contrary to the case of the Japonica type and especially the rice grain produced in the Indian districts shows this tendency.

The number of cell layers in longitudinal radius of rice grain of Indica type varieties is from 20 to 30 on the average, more than that of the Japonica type.

The number and arrangement of cell layers are determined by the genetic characteristics of strains and varieties and almost not by the change of environment at the ripening stage.

Differentiation and development of aleuron layer[6]

Around the fifth day, two or three dorsal exterior cell layers being the differentiation both in morphology and in stainability, and on the sixth or the seventh day, the cells increase from four to six layers and aleuron cell appears.

In the other parts of the grain, after the last cell division on the eighth day, the daughter cells differentiate into aleuron layers at the most external one or two cell layers. (Fig. 2)

The number of aleuron layers are slightly more abundant in lowland rice than upland rice and generally less in Indica type than in Japonica type. Especially on the dorsal surface of the grain of Indica type rice, only two or three aleuron layers exist.

On the other hand, the high temperature during the ripening period, especially the high night temperature during the differentiation stage of aleuron layer increases one or two more layers in Japonica type rice.

Since the aleuron cell differentiates specifically at the dorsal side which faces the passage

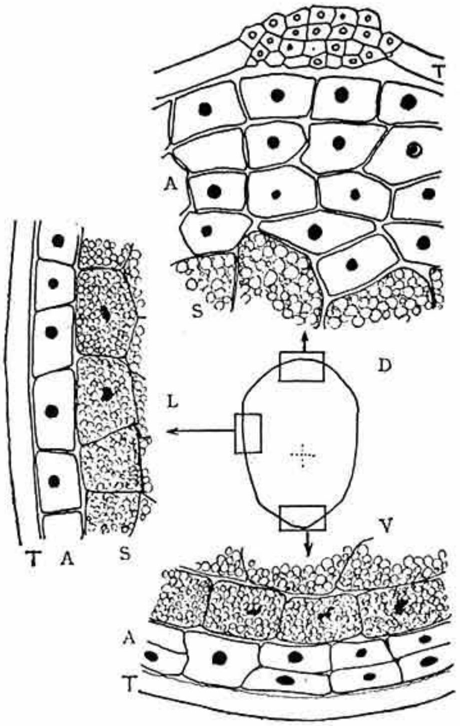

Fig. 2. Aleuron cells in different parts of the kernel (cross section)
A: Aleuron cells
D: Dorsal side
V: Ventral side
L: Lateral side
T: Testa (nucellar tissue)
S: Starch cell
(Hoshikawa 1972)

of reserve substance, it is presumed that some special function of the aleuron cell exists not only for germination process but also for ripening process.

Enlargement of endosperm cells[3, 7, 8]

The growth of rice grain depends principally upon the enlargement of each cell at the stage from the determination of the whole number of endosperm cells on the ninth day to the nearly complete formation of grain on around the 20th day.

The method of cell enlargement is determined by the position of cell in the endosperm tissue. (Fig. 3)

The cells situated on the dorso-ventral radius enlarge mainly in the direction of the dorso-ventral radius forming cylindrical body. As the enlargement of cells in the dorsal side from the central point of grain is more active than that of the ventral side, the dorsal radius becomes longer than the ventral one.

This character is recognized remarkably with the Japonica type rice especially with the "soft textured" rice produced in the Tohoku districts or in the coastal areas of the Sea of Japan. On the other hand, the cells situated on the lateral portion of the endosperm tissue develop sectorially from the central point of grain, making radial arrangement of cells.

As for the Indica type rice, the cell enlargement on the dorso-ventral radius is not so active and the enlargement develops radially from the central point of grain; however, the final number of cells is not large so the produced grains are slender. (Fig. 4)

In any portion of grain, the cells situated at the inner side of grain (formed in earlier stage) are larger and the cells of the peripheral portion (formed in later stage) are smaller. The aleuron cells located in the extreme outer edge of the endosperm cell layer are the smallest.

Progress in cell growth is dependent on the location of cells in the grain; the inner cells progress more rapidly than the cells of the outer layer, attaining to the mature size nearly on the 12th day, while more than 20 days are necessary to complete the maturity of outer layer cells.

The shape of grain in determined mainly by the degree of the growth of cells developed in comparatively early stage and located on the dorso-ventral radius of the grain; therefore, the interior and exterior environmental conditions from the fifth to the seventh day are dominant on the determination of the grain shape.

The unfavorable conditions for the growth of grain cause the grain shape to become slender due principally to the poor development

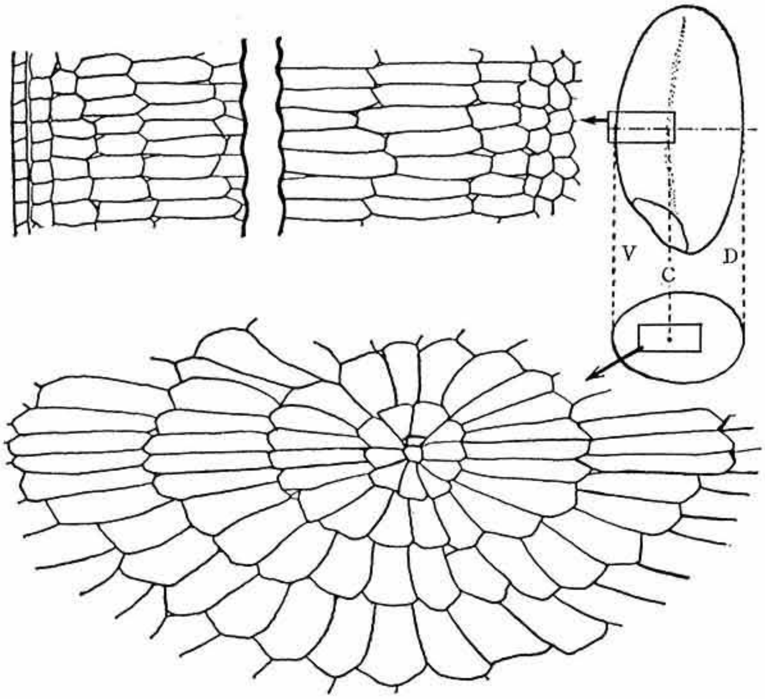

Fig. 3. Enlargement of the endosperm cells
V : Ventral side
D : Dorsal side
C : Central point (line) (Hoshikawa 1972)

in length of the interior cells of the grain than to the poor increase in the number of cells.

The high temperature during early-season culture restricts the growth in the longitudinal and lateral directions of the endosperm cells; the somewhat small and round shape of rice grain produced by the early-season culture is caused by this fact.

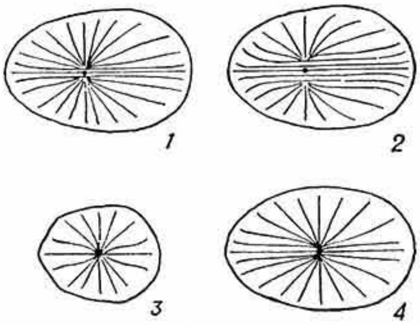

Fig. 4. Arrangement and elongation direction of cells in the cross section of rice endosperm
1 : Japonica type 'hard textured' rice
2 : Japonica type 'soft textured' rice
3 : Indica type
4 : A type of upland rice in Japonica rice
Left faces to ventral side

Mechanism introducing reserve substances into endosperm

The vascular bundles is the conducting stand of pericarp is the passage for the reserve substances into the endosperm tissue which runs along the endosperm's dorsal line.

The vascular bundle system consists of many conductive elements which are bound in a shape like the pipe bundle of a pipe organ; that is, each pipe terminates in different heights so the substances translocated upward through the pipes are discharged to the endosperm from the whole dorsal surface of the grain. (Fig. 5)

The translocated substances come into the endosperm tissue passing through the nucellar

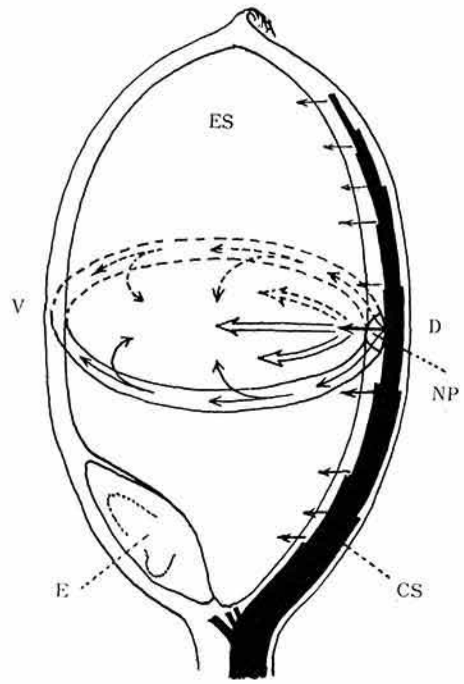

Fig. 5. Transporting route of storage substances into endosperm
C S—Conducting strand
N P—Nucellar projection
E S—Endosperm
E—Embryo
V—Ventral side
D—Dorsal side

projection. The way to the endosperm is not always the same through the whole ripening stage; at the early stage, the substance comes into the endosperm from all over its surface passing through the nucellar epidermis which surrounds the endosperm while after the 20th day, the area of inflow is gradually limited to the dorsal portion.

The substances taken up into the endosperm move through plasmodesm from cell to cell along the dorso-ventral axis in the endosperm tissue to be accumulated in cells.

The wall of the endosperm cell is well furnished with plasmodesmata which serves for the accumulation of reserve substances. The starch reserve begins at the central portion of the grain at first and at the peripheral portion especially at the ventral portion which is the most distant place from the dorsal part, starch is accumulated in cells at the latest stage.

The results of detailed ultrastructural observations on the process in which rice starch is formed as compound granule from the start stage in proplastid and then the developed proplastid differentiates into amyloplast are described in the originals[10),11),12)] together with the process of the formation of protein body with concentric zonal structure in proteoplast or protein-forming plastid.

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