Structure of rice caryopsis in relation to strategies for enhancing yield

G.A.I. Ebenezer, S. Krishnan, and P. Dayanandan

IN: Rice Research ~~for~~ ~~food~~ security and Poverty Alleviation (Eds. Peng. S and Hardy, B) IRRI, Philippines, pp 109-117. 2001.

Rice varieties with higher yield potential can be developed by increasing the number of grains per area, by increasing the grain weight, or by a combination of both. Our studies on the structure, histochemistry, grain filling, and response to plant growth regulators (PGRs) have clarified certain unique features of the spikelet of rice grains and have identified strategies required to enhance the weight of rice grains. The most important barrier to grain weight is the space limitation imposed by the fertile glumes, the palea and lemma within which the caryopsis should develop.

Experiments with intact plants and excised spikelets reveal that most PGRs, singly or in combination, do not influence the growth of glumes. Brassinoloide (BR) promotes growth of the palea and lemma when applied during the panicle initiation stage. BR at 10^{-7} M and benzylaminopurine (BAP) at 10^{-5} M applied together as a soaking spray increase grain size and dry weight by 39%. This is achieved through promotion of cell size and cell number, of both the palea and lemma, and the caryopsis within. The aleurone cells of the treated grains increase to 160,000 from 75,400 in control grains. Strategies for yield improvement could aim at increasing the dry grain weight, either by (1) breeding rice varieties with a larger palea and lemma or by (2) producing varieties that respond to PGR application by rapidly increasing the size of the palea and lemma.

A mature rice grain varies in length between 5 and 10 mm, weighs between 15 and 40 mg, and has a specific gravity of 1 to 1.2 and porosity of 60% (Houston 1972). An IR50 rice grain at the time of sowing has a length/width/thickness of 8.5/2.7/1.8 mm, respectively. The dry weight of the grain is 21.6 mg. The length/width/thickness of a caryopsis is 6.1/9.1/1.5 mm, respectively. A mature grain consists of a caryopsis surrounded by a palea and a lemma and two sterile lemmas (Fig. 1). The base of the lemma is swollen and is known as the rachilla. An abscission zone occurs below the two sterile lemmas and helps separate the pedicel from the paddy grain. The apical

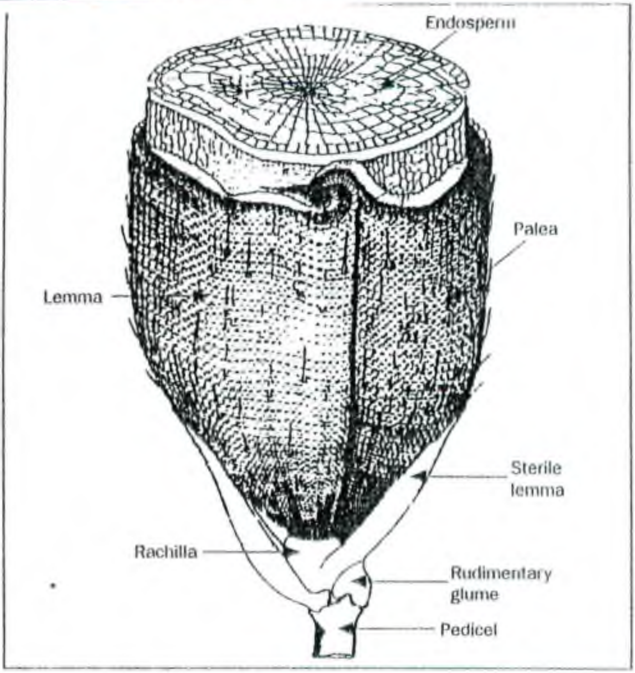

Fig. 1. Perspective diagram of a rice grain.

portion of the pedicel is considered to represent a rudimentary glume. In transverse section, the mid-region of the caryopsis shows a typical arrangement of the aleurone layer surrounding the endosperm cells. It is estimated that about 75,400 endosperm cells make up the bulk of the storage tissue.

Grain filling and transport of assimilates

During early development of the caryopsis, the endosperm and embryo are isolated from the rest of the maternal tissue by a prominent cuticular layer that surrounds the nucellar epidermis. However, nutrients are transported to the endosperm through a single ovular vascular bundle on the ventral side of the ovary (Fig. 2). Figure 3 is a summary diagram of our current understanding of the structure of the vascular region involved in the transport of assimilates into the developing caryopsis. A pigment strand and a nucellar projection mediate this transport process. Between 1 and 7 days after fertilization, nutrient material enters the endosperm through these still persistent nucellar tissues. Subsequently, solutes from the ovular vascular bundle move circumferentially into the nucellar epidermis and centripetally from the nucellar epidermis into the endosperm. The nucellar epidermis is specially reinforced with wall thickenings to provide mechanical support during this period of active enlargement of the caryopsis. A comparison of grain filling in C_3 and C_4 cereals suggests that rice has structural features intermediate between these two types (Krishnan 1996). The

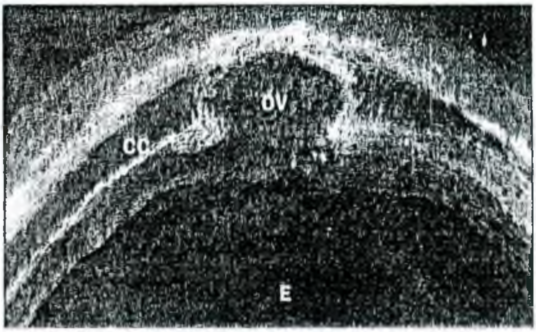

Fig. 2. Cross section of a mature rice caryopsis showing red autofluorescence of chlorophyll in cross cells. The cross-cell photosynthesis may also contribute to the assimilate that enters the caryopsis. X 250. CC = cross cell, OV = ovular vascular bundle, E = endosperm.

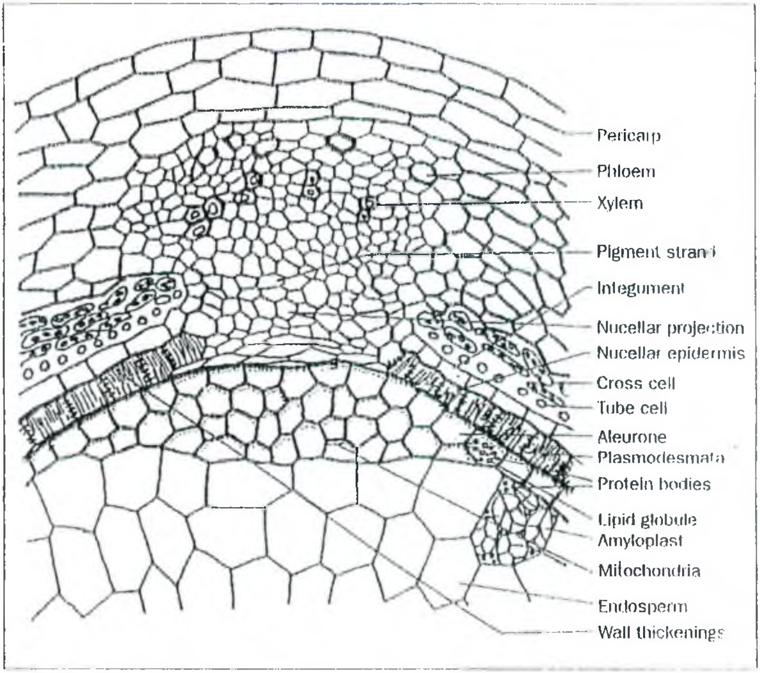

Fig. 3. Diagrammatic representation of a portion of the ventral side of the caryopsis.

situation in rice is allied to that of wheat although no xylem discontinuity is known in rice. The pigment strand and the nucellar projection in rice are not as well differentiated as in wheat. The significance of all maternal and filial structures associated with grain filling is likely to be better understood in the near future since the developmental biology of the cereal endosperm is now beginning to be analyzed with tools of molecular biology (Olsen et al 1999).

Anatomy and histochemistry of glumes

A large number of cultivars and species were examined for the organization of grain structure. The palea and lemma reach their maximum length at the time of anthesis. The outer surface of fertile glumes, palea, and lemma consists of rows of large epidermal cells with silicified knobs alternating with long, typically bicelled hairs. The apical cell of the bicelled hair is very thin and collapsible. A lemma generally has about 35 stomata on its outer surface. These occur mostly in rows along the two edges that curve around the palea. A few stomata are also found on the mucro and apiculus or awn, and the bases of sterile glumes. The stomata on the palea are confined to the apiculus. The stomatal apparatus on the lemma is about twice as large as that on a leaf. The guard cells of these stomata possess amyloplasts.

About 11 species of *Oryza* have been analyzed for the frequency and dimensions of the stomatal apparatus found on the lemma. The length of stoma ranges from 26 µm in the case of *O. eichingeri* to 32 µm in *O. punctata*. Width of the stomatal apparatus ranges from 33 to 45 µm. The largest stomatal pore is found in *O. latifolia*. Most species have stomata in a single row. In *O. grandiglumis*, however, two rows of stomata occur in the middle of both the curving edges of the lemma. In *O. granulata*, stomata occur only over the apiculus. The inner epidermis of the palea and lemma is composed of large cells, stomata, and bicelled hairs. The latter are concentrated in the apical region (Ebenezer et al 1990). Between the inner and outer epidermis are three layers of cells, two of which, located on the side of the outer epidermis, develop into long, highly sclerified fibers (Figs. 4 and 5). The layer close to the inner epidermis

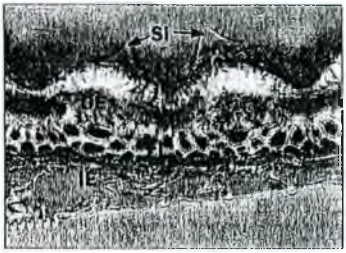

Fig. 4. Transverse section of mature lemma showing silica knobs on the outer epidermis. X 500. SI = silica knob, OE = outer epidermis, FI = fiber layer, IE = inner epidermis, Pa = parenchyma.

remains thin-walled, and along with the inner epidermis is compressed during the development of the caryopsis. Only the outer epidermis contains chlorophyll

Spikelets containing near mature grains were excised and placed in dye solutions. The inner and outer epidermal peels were then examined with a microscope. Figure 6 reveals that the bicelled hairs readily take up dyes and accumulate them in the apical cells. The Prussian blue technique reveals that water readily enters into the

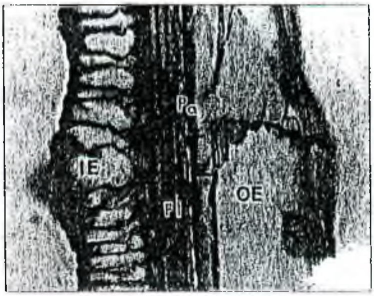

Fig. 5. Longitudinal section of lemma. X 420. IE = Inner epidermis, Pa = parenchyma, Fl = fiber layer, OE = outer epidermis.

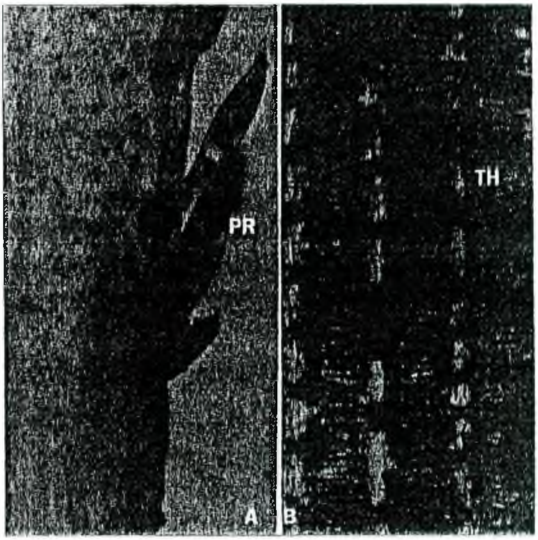

Fig. 6. (A) Inner epidermis of lemma of *Oryza sativa* cultivar Ponni stained with Coomassie brilliant blue R 250 showing protein within the bicelled hair. X 125. (B) Outer epidermis of lemma of *O. glaberrima* after transport of reagents for Prussian blue staining. The microhairs are bicelled, tricelled, and four-celled. X 125. PR = protein, TH = three-celled hair.

bicelled hairs. Such hairs are present on both the outer and inner surfaces of the palea and lemma. Interestingly, the number of cells in such hairs is not always two; three- and four-celled hairs were also observed. These hairs also accumulate amorphous protein as shown by staining with Coomassie brilliant blue reagent in IR50, as well as in cultivar Ponni and *O. grandiglumis*.

Physiology of glumes

The palea and lemma are fully differentiated at the time of anthesis and have about 1 mg of total chlorophyll per gram fresh weight. The caryopsis and flag leaf have about 0.4 mg and 3 mg of chlorophyll per gram fresh weight, respectively. The spikelet has very little silica before anthesis but rapidly accumulates silica from the day of anthesis until about 20% of the dry weight of the hull is made of silica. Water content of the palea and lemma drops from about 85% just before anthesis to 10% at the time of grain maturity.

The transpiration rate is very high in spikelets compared with that of a flag leaf. Under identical environmental conditions, the transpiration rate of the spikelet is 3.6 mg cm^{-2} h^{-1} versus 2 mg cm^{-2} h^{-1} for the flag leaf. Compared with a maximum number of about 100 stomata cm^{-2} in a spikelet, the adaxial surface of a flag leaf has about 35,000 stomata cm^{-2}. The abaxial surface of the flag leaf has about 40,000 stomata cm^{-2}. Water loss through a single stoma on the outer surface of a lemma is 1,200 times more than through a single stoma of a flag leaf. A single spikelet loses about 4 mg of water h^{-1}, whereas an entire flag leaf transpires only 60 mg of water h^{-1}. This high rate of water loss is probably an essential physiological requirement for grain filling.

Strategies for enhancing yield

The most important barrier to an increase in grain weight is the space limitation imposed by the fertile glumes, the palea and lemma, that cover the caryopsis. They provide a restricted space within which the caryopsis should develop. Matsushima (1970) showed that insertion of vinyl film inside the palea and lemma reduced the space, resulting in a correspondingly reduced caryopsis. Takeda (1973) demonstrated that the differential rate of elongation in some cultivars may produce a caryopsis longer than the length of space provided within the palea and lemma, and that such an imbalance in growth may result in a notched caryopsis. It may not be possible, however, to do away with the palea and lemma since they appear to be essential protective structures and organs that regulate the transport of water and minerals during grain filling and maturation.

Clipping the apical 2/3 to 1/2 portion of the glume interferes with the normal development of ovaries, leading to diminutive caryopsis. Covering the apical 1/3 portion of the spikelet with lanolin or plastic tubes, however, results in the development of the caryopsis (Fig. 7), although the fruit is abnormal in shape and incompletely filled. These experiments also reveal that the growth potential of the caryopsis is

Fig. 7. The apical region of the glumes was clipped and the exposed region covered with a plastic tube (PT).

limited by the space provided within the palea and lemma, thus confirming the previous observations of Matsushima (1970), Seo and Ota (1982), Takeda (1973), and Takahashi and Takeda (1971).

Rice varieties with higher yield potential can be developed by increasing the number of grains per area, by increasing the grain weight, or by a combination of both. Breeding strategies have primarily focused on increasing the number of grains, and it is assumed that the grain-weight component is recalcitrant. A large and heavier caryopsis can be obtained only when the space enclosed by the palea and lemma is larger, thus permitting the caryopsis to enlarge and fill the space. The palea and lemma, however, are fully developed even at the time of anthesis. These structures are generally insensitive to external application of plant growth regulators (PGRs) (Ebenezer 1989, Ebenezer et al 1990). Any attempt to alter the size of the palea and lemma should be carried out during the early stages of panicle initiation and not at the time of anthesis.

Table 1 summarizes investigations on the effects of different PGRs. The length, width, and thickness of control grains are 8.5 mm, 2.7 mm, and 1.8 mm, respectively. Brassinoloide (BR) + benzylaminopurine (BAP) treatment enhances grain size by affecting all three dimensions (10.6 mm, 3.1 mm, and 2.2 mm). In BR- and BAP-treated plants, the palea and lemma are larger even at the time of anthesis. Treatment also increases the number of aleurone cells from 202 in the control to 280 per cross-sectional area. The aleurone cells occur mostly in a single layer surrounding the endosperm cells. The endosperm cell number also increases from 265 per cross-sectional area in the control to 400 in the treated caryopsis. Careful microscopic analysis reveals that the total number of endosperm cells increases from 75,400 in the control caryopsis to about 160,000 cells in the treated caryopsis. The size of an endosperm cell also increases by 40% in the BR + BAP treatment.

Histochemical studies of the control and BR + BAP-treated grains reveal that an increase in grain size does not alter the pattern of distribution of the major storage

Table [illegible]. Effect of plant growth regulators on yield in IR50 rice.

Treatment[a] (M)	Fresh weight of 100 grains (g)	%	Dry weight of 100 grains (g)	%
Control	2.32	–	2.16	–
Brassinoloide (BR) 10^{-7} M	2.85	23.2	2.73	26.4
Benzylaminopurine (BAP) 10^{-6} M	2.71	16.9	2.61	20.9
Gibberellic acid (GA_3) 10^{-6} M	2.56	10.4	2.46	14.0
Kinetin (KIN) 10^{-5} M	2.73	17.8	2.67	23.7
BR 10^{-7} M + KIN 10^{-5} M	2.84	23.0	2.74	26.8
BR 10^{-7} M + BAP 10^{-5} M	3.12	34.7	3.00	38.9
BR 10^{-7} M + GA_3 10^{-6} M	2.72	17.3	2.54	17.6

[a]M = molar.
Source: Krishnan et al (1999).

materials. In both the control and treated caryopsis, aleurone cells and lipid occur mostly in the subaleurone layers. The bulk of the endosperm is made up of starch and all other storage components are similarly distributed in both grains (Krishnan 1996).

Development of the caryopsis is a postanthesis phenomenon and can be influenced only if BR and BAP are applied immediately after anthesis. Previous pot-culture experiments have shown that auxin and triacontanol-based products promote yield in rice. Of all the PGRs that we investigated, BR appears to be the most promising, especially at 10^{-7} M with 10^{-5} M BAP.

Other important strategies for yield improvement could aim at increasing the harvest index by reducing the hull proportion of grain and increasing the grain weight. In most cultivated rice, the hull proportion varies from 16% to 35% and dry grain weight from 15 to 40 mg. Can the hull proportion of grain be reduced to less than 10% and dry grain weight increased to more than 50 mg? The size and weight of a rice grain can be increased either by (1) breeding rice varieties with a larger palea and lemma (which in turn would enclose more space for the caryopsis to develop) or by (2) producing varieties that respond to PGR application by rapidly increasing the size of the palea and lemma. Such varieties can be developed either through conventional breeding or by the application of molecular techniques.

References

Ebenezer GAI. 1989. Developmental and physiological studies on the spikelet of rice (*Oryza sativa* cv. IR50). M. Phil. thesis submitted to the University of Madras, India.

Ebenezer GAI, Amirthalingam M, Ponsamuel J, Dayanandan P. 1990. Role of palea and lemma in the development of rice caryopsis. J. Indian Bot. Soc. 69:245-250.

Houston CE. 1972. Rice hulls. In: Rice chemistry and technology. St. Paul, Minn. (USA): American Association of Cereal Chemists. p 301-352.

Krishnan S. 1996. Histochemical and physiological studies on the caryopsis of rice (*Oryza sativa* L.). PhD thesis. University of Madras, India.

Krishnan S, Azhakanandam K, Ebenezer GAI, Samson NP, Dayanandan P. 1999. Brassinosteroids and benzylaminopurine increase yield in IR50 indica rice. Curr. Sci. 76(2):145-157.

Matsushima S. 1970. Crop science in rice. Tokyo (Japan): Fuji Publishing Co. Ltd. 374 p.

Olsen O-A, Linnestad C, Nichols SE. 1999. Developmental biology of the cereal endosperm. Trends Plant Sci. 4:253-257.

Seo SW, Ota Y. 1982. Role of the hull in the ripening of rice plant. IV. Morphological relationship between hull and kernel among the grains with a different degree of ripening. Jpn. J. Crop Sci. 51(3):354-359.

Takahashi M, Takeda K. 1971. Unbalanced growth between caryopsis and floral glumes in rice. Sabrao Newsl. 3:35-37.

Takeda K. 1973. A new conception on the mechanism of notching in rice kernels deduced from a viewpoint of growth rate of the developing caryopsis. II. The growth process of the developing caryopsis observed with soft X-ray. Bull. Fac. Agric. Hirosaki Univ. 20:20-28.

Notes

Authors' addresses: G.A.I. Ebenezer, P. Dayanandan, Department of Botany, Madras Christian College, Tambaram 600 059, India; S. Krishnan, Department of Botany, Goa University, Goa 403 206, India.

Citation: Peng S, Hardy B, editors. 2001. Rice research for food security and poverty alleviation. Proceedings of the International Rice Research Conference, 31 March-3 April 2000, Los Baños, Philippines. Los Baños (Philippines): International Rice Research Institute. 692 p.