Journal of Cereal Science **36** (2002) 261–284
doi: 10.1006/jcrs.2002.0435, available online http://www.Idealibrary.com on IDEAL®

Cereal Grain Structure and Development: Some Implications for Quality

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Received 5 July 2001

ABSTRACT

The grains of cultivated grasses that are conventionally described as cereals share many structural and developmental features. At the same time they are distinguished, one from another, by many individual characteristics, which fit them for different types of processing. In this review an attempt is made to describe common characteristics and to identify significant variables, both among and within species, that influence the grains' fitness for processing.

A fruit is defined by every component part, but research has been concentrated disproportionately on those components perceived as being of greatest importance. For the continuation of the species the only essential component is the embryo, but for exploitation by humans the largest component, the endosperm, is the most important. It is inevitable that this review reflects the uneven emphasis of the literature on which it is based. A further justification for the bias is the potential that endosperm offers, by virtue of its simplicity, as a model whereby fundamental mechanisms of cell behaviour and control might be elucidated. This has already been recognised by a few but benefit may result from its wider appreciation. © 2002 Elsevier Science Ltd

Keywords: development, structure, fracture, endosperm.

INTRODUCTION

In this review we describe tissues present in grains of all major cereals and define some aspects of quality that are relevant to current uses. An attempt is made to bring together observations made by microscopical and other techniques over a long period, paying special attention to developmental events in one tissue that may influence events in others. Recent advances in molecular techniques have made possible the monitoring of gene products synthesised throughout cereal grain development. This has created the opportunity to relate control mechanisms to developmental events and as a result attention has become focused on accounts of these events. Establishing relationships between control mechanisms and evnts will facilitate intervention to bring about precise improvements in quality and it is likely that most effort will be directed towards the starchy endosperm.

Grasses produce single seeded fruits, each formed from a single carpel with no special method of opening to liberate the seed. This type of fruit is known botanically as a caryopsis. The embryo and endosperm, which are both the products of fertilisation, are surrounded by adherent seed coats and pericarp tissues that formerly made up the carpel wall. Caryopses of cereals may unambiguously be called grains but the terms berry and kernel, which are often applied, specifically describe other types of fruit. They can thus cause confusion and are best avoided.

Although the components of cereal grains are adapted differently among cereal species, a generalised structure can be described for all cereals (Fig. 1).

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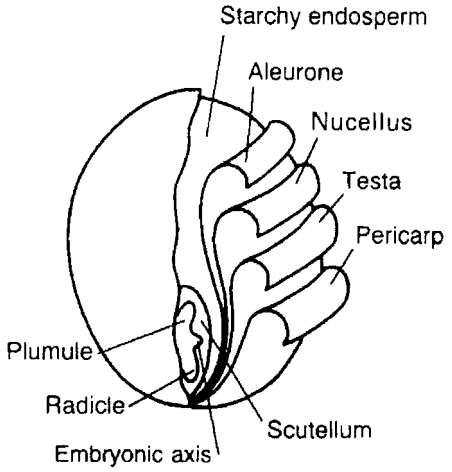

Figure 1 Generalised cereal caryopsis, showing the relationships among tissues. (Reproduced from reference 1 with permission.)

Extremely accurate structural details of examples of most cereal species can be found in Winton and Winton[2]. Unfortunately there is no equivalent recent text but some of the Wintons' drawings are reproduced in Kent and Evers[1]. O'Brien[3] presented an historical review of structural studies while a useful synopsis of literature relating to caryopsis anatomy up to its date of publication appeared in 1973[4].

GRAIN STRUCTURE

Grain shape and size

Shape, size and mass are the most readily identifiable characteristics of the fruits of individual cereal species. Thousand grain weight ranges between 0·14 g for teff (*Eragrostis tef* (Zucc.)) to 600 g for maize[1]. However, within species there is considerable variation, and morphology can be associated with quality parameters. In rice, the ratio of length to breadth is a useful guide to the nature of the endosperm, and more significantly the starch type present. Shorter grains tend to be associated with stickier cooking quality. Maize grains vary from the near spherical popcorn to flattened and angular flint maize. A depression on the distal face arising through contraction of endosperm is characteristic of dent maize and reflects the presence of a region of soft endosperm within a harder textured cup. Caryopses of members of the tribes Triticeae (wheat, rye, barley and triticale) and Aveneae (oats) can be distinguished by the presence of a crease, a re-entrant region on the ventral side, extending along the grain's entire length, and deepest in the middle. It is most marked in wheat (e.g. *Triticum aestivum* L. and other species e.g. *T. durum*, *T. compactum*), followed by triticale (*Triticosecale* ssp Wittmack), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.). In milling to produce white flour, the crease presents the greatest difficulty for separation of starchy endosperm from other tissues.

While differences among species are sufficiently great for reliable discrimination by machine vision systems such as the Foss-Tecator Graincheck 312[5], the lesser variations in shape and size occurring among cultivars are currently useful only when the judgement and discretion of trained personnel are brought to bear.

Within a cultivar, sources of variation in grain size include the growing conditions of the crop, the grains position within the inflorescence. In general the largest grains occur in the centre of the inflorescence[6] and, in species with multiple tillers, the mean grain size decreases progressively from grains borne on the main culm.

It is important to distinguish between inherent grain size as a varietal or positional characteristic and size reduction, or shrivelling, due to poor plant nutrition or disease. Shrivelling is almost always a manifestation of poor endosperm development, while small, plump wheat grains produced on small-grained varieties have proportions of endosperm equal to those of equivalent grains from large-grained varities. Some of the best milling wheat types such as the hard classes grown in North America have characteristically small grains. While grain filling is largely a function of successful starch accumulation, it is also found that, within a sample of wheat, the proportion of storage proteins is greater in larger caryopsis[7,8]. A positive relationship between grain size and *alpha*-amylase activity[9] was suggested based on analysis of data relating to an extended period during which both wet and dry harvests occurred[10].

Irrespective of the size or shape of individual grains, the uniformity of their morphology is important in terms of processing. More uniform wheat samples give more predictable milling performance and malting performance of barley relies heavily on homogeneous samples. This is true for all the relevant grain parameters such as protein but is most readily achieved through sorting of grain by physical parameters such as degree of shrivelling[11].

Grain components

Embryo

The embryo arises from the fusion of male and female gametes. It is the most important grain component for the survival of the species as it is capable of developing into a plant of the filial generation. At grain maturity, it comprises an embryonic axis (shoot, mesocotyl and radicle) and scutellum, which is considered to be homologous with a cotyledon. The name scutellum derives from its shield-like shape and it lies between the embryonic axis and endosperm. The embryo has the highest concentration of lipid and hence lipid-soluble vitamins in cereal grains. It also has the highest moisture content in the mature grain[12] but not all water-soluble vitamins have the highest concentration here[13]. With the exception of maize, where the oil is economically important, the embryo is not usually identified as a target for improvement by breeders.

Endosperm

Endosperm consists of two tissues, starchy endosperm and aleurone. Starchy endosperm occurs as a solid mass occupying the centre of the grain and many references to 'endosperm' actually refer only to this component. It is the largest morphological component in all cereals and it is also the component with the greatest value. It has been conjectured that it is by virtue of the endosperm being the largest tissue rather than it having a superior composition, that cereals provide the greatest contribution to animal (including human) nutrition – the principal purpose for which they are cultivated[14].

Aleurone cells are block-like, with thick walls and prominent large nuclei, and they occur in one or more (according to species) continuous layers surrounding the starchy endosperm and, in modified form, the embryo. They have relatively high concentrations of protein, lipid, vitamins and minerals. Aleurone cell size is not a function of grain size; in wheat they are approximately 50 μm cuboids but in the much larger grain of maize they are smaller. Pigmentation in the aleurone layer can give grains of some cereals a blue, red or almost black appearance. The cell wall of typical aleurone cells becomes uniformly thick and distinctly two layered at maturity in wheat, barley, rye, and oats[15]. Aleurone cell walls in a number of cereals have high concentrations of ferulic acid leading to a pronounced autofluorescence under appropriate lighting conditions[16–18]. The majority of aleurone cells of each species are uniform in size and shape; however, morphologically distinct types have been described. In foxtail millet (*Setaria*)[19], aleurone cells adjacent to the placental vascular bundle are columnar in shape with ingrowths on their walls. The ingrowths aid transfer of substances from the developing embryo and endosperm. These cells are called 'transfer aleurone cells' and have also been reported in Japanese millet (*Echinochloa utilis* (L.))[20] and maize (*Zea mays* L. ssp mays)[21] but are absent in wheat[20] and rice (*Oryza sativa* L.)[22]. In caryopses where a crease is present, the cells in the ventral region surround a cavity and become distorted and crushed. They have been described variously as 'modified aleurone cells'[23], 'thick walled cells'[24] and 'groove aleurone cells'[25,26]. In wheat the 'groove aleurone cells' have pitted and irregularly thickened walls and there appears to be more than one layer, even though this is not the case for the rest of the grain. Plasmodesmata are few as compared to aleurone in the dorsal and flank regions of the grain[26].

Most of the aleurone layer is removed as part of the bran during roller milling. The removal of the aleurone layer may be less complete when decortication rather than roller milling is employed as in production of pearled barley, milled rice, 'degermed' maize, and wheat milled by processes involving abrasion in the early stages[27].

Essentially, mature starchy endosperm comprises cells in which starch granules are embedded in a matrix of storage proteins. These two components, together with cell walls and minor constituents, accumulate as a source of nutrients for the accompanying embryo when it begins to germinate. The nutrients are made available as a result of hydrolysis by enzymes produced in the embryo itself or in the aleurone, or both. Starchy endosperm cells are relatively uniform in that their components are the same. However, gradients exist to the centre of the grain (or the cheeks for grains with creases) whereby starch concentrations increase as the distance from the periphery increases. In wheat these cells have comparatively thin walls and can be subdivided into three populations: peripheral cells, prismatic cells and central cells. The peripheral cells occur as a single sub-aleurone layer and are approximately 60 μm in diameter. Beneath this layer lie the prismatic cells, which radiate in columns from the sub-aleurone cells. These cells are 128–200 μm long and 40–60 μm wide[23]. Neither they nor the sub-aleur-

one cells appear next to the modified aleurone cells in the crease region[28]. The final type of starchy endosperm cell is found in the centre of the cheeks of the wheat grain. Cells of this type are rounded or polygonal in shape with dimensions 72–144 μm long and 69–120 μm wide. The composition of cell walls differs among cereals. Rice is unique among cereals in having large proportions of cellulose present. Barley and oats have cell walls rich in mixed linkage β-glucans, while wheat has arabinoxylans as major components[29]. Little information exists as to the possible significance of these differences in relation to physical properties but the β-glucans are claimed to have beneficial effects on cholesterol metabolism in consumers[30].

Tissues surrounding the endosperm and embryo

Nucellus The maternal tissue immediately surrounding the endosperm and embryo is the nucellar epidermis. The compressed epidermis with its thin outer cuticle is all that remains of the nucellus, the mass of tissue in which the endosperm and embryo developed. It is particularly prominent in sorghum (*Sorghum bicolor* (L.) Moench), but is apparently absent from mature oat grains, and in maize only the cuticle persists. The nucellar epidermis is also called the hyaline layer or perisperm and it is regarded as a seed coat. Between the nucellar layer and aleurone in wheat is an amorphous layer, which is thought[31] to be analagous to the nucellar lysate layer found in developing barley[32]. It is probably the remnants of the nucellar cells once present between the epidermis and the embryo sac.

A mass of nucellar tissue persists in the region of nutrient flux from the vascular supply and, in grains with a crease, it takes the form of a column: the nucellar projection, lying adjacent to the inner extremity of the crease.

Testa The true seed coat is the testa or spermoderm. Its inner face lies adjacent to the cuticle of the nucellar epidermis where present. The cuticle of the testa is thicker than that of the nucellar epidermis and it comprises two layers in wheat, rye and triticale, one layer in barley, oats, and rice and, as with the nucellar epidermis, only the cuticle remains in maize. In mature sorghum, no testa is present. If present in pearl millet (*Pennisetum glaucum* (L.) R. Brown) it is inconspicuous, as is the nucellar epidermis. When present, the cuticle of the testa is thought to be responsible for the relative impermeability of the grain to water over most of its surface. Pigmented corky substances may accumulate late in development. When present they confer a 'red' characteristic on grains and contribute to impermeability. There is also a strong correlation between degree of red pigmentation and resistance to preharvest sprouting. In transverse sections of caryopses featuring a crease, a discontinuity in the testa can be seen in that region. During grain development this facilitates transport of nutrient solution from the vascular strand to the nucellar projection and thence to the endosperm. As the grain matures, impermeable material accumulates to form a cylindrical plug, the pigment strand, between the borders of the integuments, completing the waterproof layer around the grain. In red wheats, the pigment strand is dark brown but in white wheats it is less obvious and much paler. In maize, which does not have a crease, the tip cap area is the point of entry for nutrient transport from the plant to the developing grain. This path is sealed during maturation with a dark layer of dense cells called the hilar layer which may be a possible source of black specks in maize meal. This tip cap is the weakest point of the mature maize grain and is the point of water ingress when grains are steeped or tempered[33]. The two integuments of the carpel, from which the testa derives, are leaf-like structures that surround the nucellus. They originate from the chalaza, close to the point of attachment, and enclose the developing grain. Close to the tip of the embryo, where the integuments fail to meet, a pore – the micropyle, exists, through which water can penetrate. When a grain is steeped in water, the water enters most rapidly through the micropyle, hydrating first the embryo and then the endosperm progressively more distant from it. In an unsaturated grain such a moisture gradient is maintained. In wheat, both cellular layers of the testa are considered to develop from the inner integument[34]. However, the long axes of cells in the two layers lie at right angles each to the other. The inner layer lies at right angles to the crease where it attaches to the pigment strand while the outer layer runs parallel to the crease in the same region. The angle of both layers to the long axis of the grain becomes more oblique as they move away from the crease region[28].

Pericarp Those tissues of the caryopsis lying outside the seed coats originate as components of the carpel wall. They are thus parts of the fruit but

not of the seed. They vary considerably among species but they are generally dry empty cells, some maintaining their shape and the integrity of layers, others being shrunken and distorted, leaving large, frequent intercellular spaces. The innermost fruit coat is the inner epidermis of the pericarp. It is an incomplete layer comprising isolated groups of wormlike cells, the shape of which inspires the description tube cells, by which they are usually known. The long axes of tube cells lie parallel to the long axis of the embryo.

Outside the incomplete tube cell layer lie cells that are said to be unique to grasses, the cross cell layer, so called because the long axis of the cells lie at the right angles to that of the embryo. In immature grains chloroplasts are present in these cells as they are in tube cells also. Cell shape varies among species and in different areas on the same grain. Over most of the surface of wheat, barley, triticale and rye they form of a complete layer of cells about six times as long as their width, arranged in rows, but at the grain tips they become more square[2]. Because of the fragmentary nature of the tube cell layer, it is the cross cells that adhere to the underlying cuticle of the testa. In maize, rice, sorghum and pearl millet there are large spaces among the distorted, more elongated cells. In oats, tube cells and cross cells are indistinguishable from other inner pericarp cells, which are all elongated and distorted with no common orientation. In rice, other pericarp cells follow a cross cell orientation.

Grains at an early stage in development may have a green pearly appearance and this is due to the chlorophyll in the inner pericarp cells, partly obscured by tiny starch granules that reside temporarily in the parenchymatous cells lying between inner and outer epidermis. In most species, this tissue autolyses towards maturity, with the products of digestion being translocated to the still expanding endosperm. Sorghum is exceptional in that the mesocarp, several layers thick, and containing starch granules, remains at maturity. In wheat, the loss of the parenchyma breaks the continuity between cross cells and outer pericarp layers which are then attached firmly only in the crease region, where the parenchyma remains intact. Isolated remains of parenchyma cells persist as intermediate cells, a discontinuous and fairly insignificant remnant of a multilayered tissue. The outer pericarp comprises the single outer epidermis, with its thin cuticle, and one or more layers of hypodermis. Although it probably helps to control water loss during development, the cuticle of the outer epidermis in harvested wheat grains does not prevent water from penetrating easily. Absorption of water emphasises the discontinuity between inner and outer pericarp as the outer layers expand and become easily removed by gentle abrasion as straw coloured membranous flakes that are described by some as 'beeswing'. The empty cells of the pericarp hold enough water to increase grain weight by 4–5% after only a few minutes' immersion[35]. They act as a reservoir for water that may ultimately enter the grain slowly through the micropyle. The absorbent nature of the pericarp is of great benefit in the milling of wheat, which is 'conditioned' or 'tempered' by water addition prior to milling. The grain is allowed to absorb water over a period of 12–24 h during which time the bran layers are toughened, enabling them to be easily removed. Water reaching the starchy endosperm renders it more mellow or friable, allowing for better milling performance[36]. Water enters at different rates in different varieties[37], penetration rate being greater in soft wheats than hard and possibly being influenced by bran composition[38]. Conditions of tempering should thus be optimised for individual grists.

Cells of both epidermis and hypodermis are elongated, with axes running in the direction of the embryo axis, except in rice, where they lie at right angles to the embryo axis (i.e. in the cross cell orientation). The hypodermis of dent maize and flint maize has 10 or more layers, pop corn (*Zea mays* L. ssp mays var everta) has 8 to 10 and sweet corn only 2 or 4. All elements of the seed and fruit coats exhibit some variation according to the position of occurrence on the grain surface. The proximal (embryo) end is the point where the grain was attached to the plant and at the distal end of wheat, rye, barley, triticale and oats epidermial cells exhibit a particular and significant specialisation in that they extend into long pointed hairs or trichomes. Before fertilisation extended trichomes cover the entire surface of the carpel, forming the feathery stigma. Expansion of the growing grain, including production of new epidermal cells towards the proximal end, concentrates the hirsute cells to the non-embryo end of the grain where the form a 'brush'. Trichomes are rich in silicon, hollow and, in wheat, approximately 0·5 mm long. Their length and surface spiral pattern is characteristic of each cereal species[39]. In many varities of oats, trichome coverage can be extensive, covering the whole surface of

Table I Tissue layers present in grains of different cereal caryopses (based on reference 2)

Cereal	Fruit coats					Seed coat	Nucellar epidermis	Aleurone
	Epidermis	Hypodermis	Intermediate layer	Cross cell layer	Tube cell layer			
Barley	√	√	√	2	√	1	√	2–4[d]
Maize	√	√	√	√	√	[a]	[a]	1
Proso millet (*Panicum miliaceum* L.)	√	√	√	√	√	[a]	[a]	1
Oat	√	√	√	√	√	1	√	1
Rice	√	√	√	√	√	1	√	1–3[d]
Rye	√	√	√	√	√	2	√	1
Sorghum	√	√	√	√[b]	√	0	√	1
Triticale[c]	√	√	√	√	√	2	√	1
Wheat	√	√	√	√	√	2	√	1

[a] A cuticular skin persists to maturity.
[b] Incomplete layer.
[c] Notional.
[d] In barley the variation is varietal while for rice it occurs within a single grain. In rice aleurone layers are more abundant in lowland than upland rice and generally less in Indica than Japonica type but the number can increase in Japonica if night temps are high at differentiation[42].

the groat. The feature has been manipulated by breeders, however, so that in some cases only the distal end is covered[40].

Work on the bubble structure of wholemeal breads has indicated that fractions of wheat bran containing cells bearing trichomes have a detrimental effect, greater than other non-endosperm fractions, on loaf volume[41]. Scanning electron microscopy showed trichomes puncturing air cell walls and thus adversely affecting crumb texture and loaf volume.

A summary of the tissue layers present in different cereals is presented in Table I.

During grain growth, nutrients are translocated from the leaves and roots of the parent plant through conducting tissues. Vascular structures are also found in the pericarp. In wheat, ryegrass, oats and bromegrass it has been found that xylem in the grain is not continuous with that in the floral axis. Rather the files of tracheary (primary xylem) elements converge upon a core of thick walled cells in the attachment region. Many transfer cells and sieve elements are located around the thick walled cells. Zee and O'Brien[43], who discovered the discontinuity, suggest that it serves to control solute flow, and that it is likely to be present in other grasses.

Lemma and palea All cereal caryopses are subtended on their parent plants by a pair of glume-like bracts known as the lemma and palea, which, in most types, surround and protect the immature fruit. When ripe, the caryopses of some species are shed from the plant free from these structures, but in other species the lemma and palea remain as a hull, in close contact with the caryopsis even after it separates from the plant. Although the hull of all hulled species is formed from the lemma and palea, the mechanism by which they remain in contact with the fruit varies. The hulls of oats and rice are held in place by structural devices (the lemma and palea in rice have a rib and groove arrangement at their meeting margins) and it is possible, by use of appropriate machines, to free the fruits from them. By contrast, the lemma and palea of barley adhere to the pericarp[2] and cannot be removed cleanly from the caryopsis by mechanical means. Naked oats have been bred, in which hulls are less tightly adhering to the grain and thresh free during harvesting[44]. Hull-less varieties of barley are also cultivated but the crop for malting purposes requires varieties with closely adhering hulls which do not detach during mashing[11].

It is usual for hulled grains to be traded with hulls in place and for analytical and compositional data to be presented for the grains as traded. In Table II, grain weights and the contributions of the main morphological components, are presented. For hulled types, proportions are given for both hulled grains and caryopses. It is notable that the proportion of the grain represented by the

Table II Grain weights and typical proportions (%) of grain parts in some cereals (based on information in reference 1). Values in parentheses are proportions of grain excluding hull

Cereal	Grain weight (mg)	Hull	Pericarp and testa	Aleurone	Starchy endosperm	Embryo	
						Embryonic axis	Scutellum
Naked grains							
Wheat	27–50	—	8·5	6·7	82	1·3	1·5
Maize	150–600	—	6·0	2·7	77·8	1·5	12·0
Rye	15–40	—	10·0		86·5	1·8	1·7
Sorghum	8–50	—	7·9		82·3	9·8	
Proso millet	n/a	16·0	3·0	6·0	70·0	5·0	
Hulled grains							
Rice	n/a	20·0	4·8 (6·0)		72·7 (90·9)	1·0 (1·2)	1·5 (1·9)
Barley	32–36	13·0	2·9 (3·3)	4·8 (5·5)	76·3 (87·0)	1·7 (1·9)	1·3 (1·5)
Oats	n/a	25·0	9·0 (12·0)		63·2 (84·0)	1·2 (1·6)	1·6 (2·1)

n/a, not available.

embryo varies, with wheat, barley, oats and rice all having smaller embryos relative to the total grain mass while sorghum, maize and millet all have larger embryos.

Germination

Germination is initiated when a viable grain imbibes sufficient water. It is the stage of the plant life cycle at which the resting embryo begins to produce functional roots and shoot. To support the embryo's early growth, reserves stored in the endosperm are solubilised by hydrolytic enzymes, in which condition they can be assimilated into the new plant. The hydrolytic enzymes are exploited in the process of malting for which large quantities, particularly of *alpha*-amylase, are desirable and, in much smaller quantities, they are beneficial also in breadmaking. However, when activity is excessive, doughs become unworkable as a result of starch hydrolysis and produce bread with sticky crumb due to the presence of undesirable dextrins. The process is dependent on the embryo being viable and still attached to the grain[11]. Good barley for malting should have a germinative capacity (GC) of greater than 96% and a geminative energy (GE) of greater than 96%. In the western world, barley is the cereal most commonly used in malting, but in Africa, sorghum is also used.

In some cereals the ability to germinate is achieved before harvest and, under wet conditions germination can begin while grains remain on their parent plants. Such undesirable behaviour is called premature germination or sprouting. Sprouting in the field is a real problem in countries where wet harvests occur. A related but less serious problem arises in wheat from *alpha*-amylase produced in the crease aleurone, late in grain maturation (late maturity *alpha*-amylase). High levels of enzyme from this source can arise even in dry conditions[45].

Another problem of breadmaking quality associated with the embryo is caused by the tripeptide glutathione. Wheat germ contains 0·345–0·460% glutathione[13] and its inclusion in flour and the resultant higher levels of reduced glutathione have been related to a detrimental effect in baking quality[46]. Typically it decreases 'Resistance' and 'Extensibility' measured by Extensograph and 'Consistency' by Farinograph[47,48]. Its use as a flow-modifying agent in pasta production has been suggested[49].

Grain processing

All cereal grains are potentially sources of nutrients for humans and livestock. They serve mainly as a source of energy, by virtue of the high starch content of their endosperm component. Methods by which energy is made available vary with cereal species, and with the community or livestock type benefiting from them. For nutrition of humans and farm stock, the starch needs to be modified by heating in the presence of water, or digested by enzymes before it can benefit the consumer. In general, human preference demands that the endosperm be concentrated by removal of most of the other grain components. In the case of

rice, most is consumed as whole endosperm, after removal of hull, pericarp and embryo. Other cereals are also consumed as entire endosperm but such practice accounts only for a small proportion of the total. With wheat, maize, rye, triticale, sorghum and millets, refinement is usually combined with reduction of endosperm to fine particles (say <150 μm) in the case of flour, or coarser particles in the case of grits or semolina. Further refinement may be carried out to concentrate individual endosperm components such as starch and protein (often called gluten, even when isolated from species that contain no true gluten proteins). Grains of most cereals find some food application after crushing and this is the most frequently applied dry treatment of oats, after removal of hulls but with pericarp/testa and embryo in place. The high oil content of oat endosperm demands that a roasting stage be incorporated to inactivate enzymes and thus prevent rancidity. When used to benefit livestock, cereal grains may be fed whole or crushed or ground without separation of components. Feed provides the main outlet for bran and other offals derived from components separated from endosperm during refinement. The main food use of barley lies in the production of malt, mainly for use in beer and spirit production. For this purpose, it is advantageous to use the entire hulled grain as the hulls provide a useful filter medium when soluble products of fermentation need to be separated from remaining solid components. Malting and fermentation can be applied to all cereal grains but brewing uses of most cereals more frequently involve addition of flour or grits as adjuncts to barley ferments. Rice fermentation to produce saké relies on the use of microbial enzymes.

Industrial separation of endosperm from other grain components is usually described as milling, and textural properties of the endosperm are particularly important where dry milling plays an important part in the transformation from grain to food. Traditional definitions of endosperm texture have been as 'hard' and 'soft' and categorisation has been entirely dependent on the method used, with probably the most widely applied methods employing particle size analysis of stocks milled by a putatively standard procedure, or a near infrared reflectance analogue. Hard endosperm characteristics are often manifested by visually recognisable features and descriptions such as vitreous, translucent, steely or horny are applied, while soft endosperm texture has also been described as floury, mealy or opaque because interfaces between voids and solid components in the softer, weaker regions scatter light. Visual characteristics are not always a reflection of hardness but differences in appearance and fracture properties between the two endosperm types can be related to the size and proportion of air spaces present. The proportion of air space in turn determines the density, with hard endosperm regions having fewer air spaces and thus being denser. The relative proportions of horny and floury maize grains depend greatly on the type of maize. Of the five main types, four (dent (*Zea mays* ssp mays var indentata), floury (var amylacea) flint (var indurata) and popcorn (var everta)) have common names associated with endosperm texture; floury and flint maizes are both named after the predominant endosperm texture found in their grains while dent maize is so called because the central floury portion of the endosperm collapses during grain drying. Popcorn is a variation of flint maize in which the dense endosperm texture leads to a build-up in pressure during cooking, resulting in the rapid expansion of the hila at the centre of the starch granules and the 'popping' of the grain[33]. In a similar way to maize grains, the density of the endosperm in rice can also vary within a caryopsis and in this case the less dense regions are called 'chalk'. The degree of chalkiness in rice depends on both variety and the maturity of the grain at harvest.

The fracture properties of endosperm are of importance, for different reasons depending on the milling process used. Because it is desirable that rice endosperm remains intact, weaknesses present in chalky areas or cracks are undesirable as they can lead to broken grains[50]. With other grains the textural properties required depend upon the end use envisaged. Soft wheat endosperm tends to fracture randomly to produce irregular particles while a higher proportion of fractures in hard wheat endosperm follow cell boundaries and produce pieces of endosperm cells where the starch granules are still bound to the protein[51]. For starch or biscuit flour production, fragmentation through the application of minimum force is desirable because a consequence of multiple roll passages or high roll pressures used in reducing hard endosperm is the creation of relatively high levels of starch damage. Starch damage involves molecular disruption of amylopectin and has the effect of increasing the capacity of starchy milled products to absorb water, a characteristic that is valued in

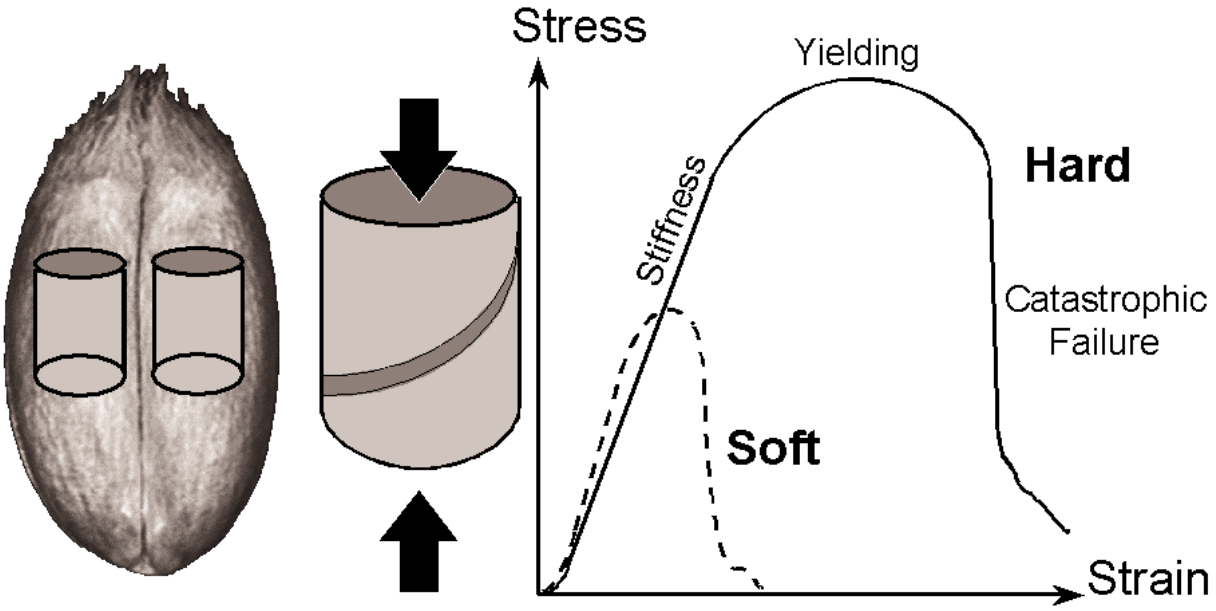

Figure 2 Schematic representation of stress–strain curves for turned cylinders of wheat endosperm subjected to terminal pressure as shown. The left hand diagram shows the relationship of the cylinders to the whole grain. (Figure reproduced by permission of CCFRA.)

flours destined for plant bread manufacture. For production of durum wheat semolina and maize grits, the requirement is for endosperm that is capable of being reduced to coarse particles with minimal accompanying production of fine particles including free starch granules. Similarly when milling barley to produce semolina, as in North Africa, steely grains are required[52].

Recently, attempts have been made to define textural qualities in authentic engineering terms. Hardness of metals is generally agreed to be their resistance to permanent deformation[53]. This concept may also be applied to food materials on the basis that most tests for the measurement of hardness or firmness result in permanent indentation or deformation of the material tested[54]. For cereals, hardness can be understood in terms of stiffness, which for cellular objects is related to density. The strength of the material is the force at which it breaks, while toughness is a measure of the degree to which a crack is arrested. A number of groups have worked in this area[55–57] demonstrating that the concept of 'hardness' can be explained to some degree in terms of accepted material properties. By using cylinders and rectangular blocks of intact endosperm, the last of these studies showed that a hard wheat (cv. Mercia) had a higher failure stress, a higher failure strain and a higher failure energy than a soft variety (cv. Riband)[57] (Fig. 2).

Fracture tests were also carried out using a wedge to induce cracking in endosperm blocks which showed that cv. Mercia yielded when it fractured and was therefore tough, while cv. Riband was found to be brittle. It was also confirmed that the less dense regions of the endosperm were mechanically less strong and less tough than the dense regions. In addition to these studies, gas adsorption (a technique used to measure the surface area of materials) was used to characterise not only flour milled from these two varieties but semolina and whole wheat also. In each case, the cv. Riband gave higher surface areas than Mercia, indicating the presence of greater void volume in the whole wheat, and smaller particles in the ground material.

In considering the differences in manner of fragmentation between hard and soft endosperm reference has been made to possible variation in cell walls. Greer *et al.*[58] found that hard wheat endosperm particles consisting of entire cells frequently had fragments of cell wall attached, suggesting that the contents played a greater role in maintaining cell integrity than the walls. However, in wheat, arabinoxylans and the related compounds (ferulic, diferulic and *p*-coumaric acid)

have also been implicated, in that Hong *et al.*[59] found evidence to suggest that for North American wheats the variation in arabinoxylan content contributed to endosperm texture. Also in durum wheat a good marker for the degree of separation between the starchy endosperm and aleurone was the concentration of diferulic acid, measured by HPLC[60]. Lempereur *et al.*[61] investigated the effects of variety and growing conditions on arabinoxylan and ferulic acid content of durum wheat and also investigated the relationship between these compounds and the ease of separation of the bran from the endosperm uring semolina milling[60]. It was found that the proportion of *p*-coumaric acid in the whole grains was positivelly correlated with the amount of bran produced during milling and could thus be used as an indicator of extraction rates. It was recognised, however, that ferulic acid would be more easily measured than the other phenolic compounds because ferulic acid autofluoresces at 420 nm when irradiated with ultraviolet light at 350 nm[25]. Analysis of the intensity of fluorescence for a transverse slice of the grain showed that it was negatively correlated with the diferulic acid content (measured by HPLC) in the final semolina. There was, therefore, some evidence to suggest that the fluorescence is somehow linked either to the degree of separation between the bran and endosperm or to the integrity of the bran. Because of its greater concentration in aleurone than starchy endosperm cell walls, it was also suggested that ferulic acid might be used as an indicator of the purity of semolina[18]. Fluorescence microscopy had previously been suggested as an indicator of the presence of peripheral non-endosperm tissues in white flours and semolinas[62,63].

Bran texture

The physical structure and strength of wheat bran is also a factor when assessing the yield of material from endosperm origins by dry milling. In fact the name 'dry milling' in this context is something of a misnomer as the addition of a few percent of water to the grain some time before milling ensures that the bran is sufficiently hydrated to toughen it enough to prevent it from shattering and contaminating the purified endosperm. Recent work on isolated bran strips confirmed that the failure strain of bran increases at higher humidity[57]. Additionally bran was found to be notch insensitive, i.e. small flaws were not easily propagated under stress. The toughness (the resistance to failure at a notch) of the bran also increased with increasing humidity and in the case of a soft UK variety, Riband, a threefold increase in the toughness was observed at greater than 80% humidity. This conflicts with work by Glenn and Johnston[64] who found bran to be toughest at 70% relative humidity, possibly a reflection of the different varieties used in these two studies. Barley bran is quite different from wheat bran in that it poses significant problems during milling due to its brittle nature. Thus, not only must the barley be pearled prior to milling to remove the husks but the bran must also be removed to prevent shatter during milling[65].

The crease in relation to processing

The standard method of flour milling involves a first stage in which grains are split open, so that the bran can be flattened and progressively scraped clean of endosperm from its inner face. The evolution of such a system owes much to the presence in wheat of the ventral crease. Were this area of inaccessible bran tissues not there, removal of non endosperm tissues by decortication, as performed in rice milling, may well have been adopted as a standard first stage of flour milling. Indeed, with the application of advanced conditioning regimes, decortication has been incorporated into recently introduced systems[66]. Investigation into the effect of crease shape on conventional milling performance showed that wheat grains with a closed crease tended to require a greater force to be applied for failure to occur than those with more open creases. However, the crack in the former case tended to be across the cheeks of the endosperm, causing more damage and, therefore, a higher release of endosperm at first break. Conversely grains with a more open crease showed cracks occurring along the crease which was thought to be preferable as it was postulated that this would cause the bran to remain as larger pieces[57].

Whereas, elsewhere in the grain, the endosperm adheres peripherally to the surrounding tissues, in the region of the crease, a void (the endosperm cavity) exists between the endosperm and the outwardly adjacent tissue. In conventional roller milling, the scraping of the bran, as described above, is controlled in such a way that the plane of separation is between the outer starchy endosperm

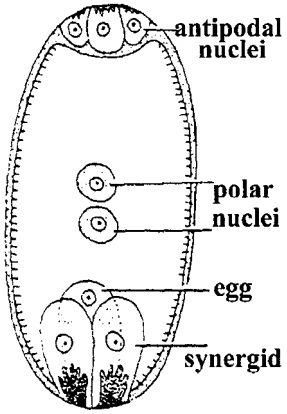

Figure 3 Diagram of the mature embryo sac (female gametophyte) (modified from reference 71).

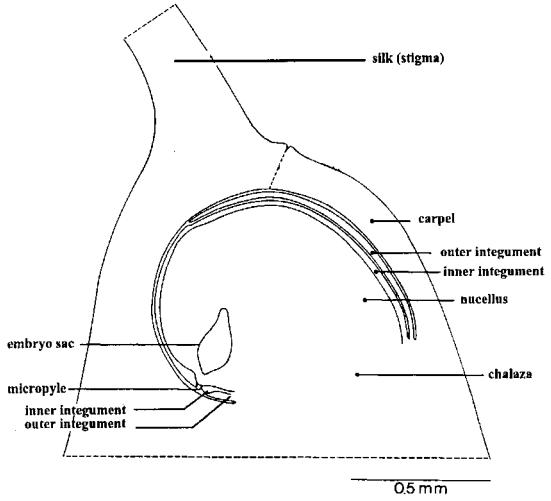

Figure 4 Median section of a mature ovary with a part of the silk of *Zea mays*. (Reproduced from reference 72 with permission.)

and the aleurone layer. While this process works efficiently where aleurone adheres to the seed coats, the unsupported aleurone adjacent to the cavity is less likely to be separated from the adjoining starchy endosperm. Large grains have a propensity to develop larger endosperm cavities[67] so the size of grains can have an influence on the amount of aleurone tissue included in flour[10]. The larger cavity found in the bigger grains compensates for the reduced surface area to volume ratio and offers a possible explanation for the observation that smaller grains contain proportionally no less starchy endosperm[68]. In U.K. samples harvested in 1995 (a dry harvest), it was found that the enzyme produced was predominantly of the late maturity type[70].

GRAIN DEVELOPMENT

General

As in higher plants generally, seeds of cereals are produced by sexual reproduction, involving fusion of male and female gametes. Gametes are produced, by the process of meiosis, in male and female structures (gametophytes) that have only a single set of unpaired chromosomes in each nucleus, a condition described as haploid. The gametes are thus also haploid. In higher plants the male nucleus lies within a pollen grain. The female gamete, the egg, is located in the female gametophyte, or embryo sac, the structure of which is shown diagrammatically in Figure 3.

Inside the embryo sac lies the egg nucleus, which, with two 'synergids' forms the 'egg apparatus'. Other structures present in the embryo sac at this time are the central cell, containing two polar nuclei and, at the micropylar end, three antipodal cells.

The embryo sac lies within a central vacuole, a void in a mass of cells known as the nucellus, which in turn is bounded almost entirely by integuments within the carpel. Other carpel structures are the stigma and style. In most cereals the style is feathery but in maize the styles are extended into long strands, which hang together out of the female inflorescences as 'silks'.

Fruit coats and seed coats originate as tissues present in the carpel at the time of fertilisation (for details or origin and development of individual components see Evers and Bechtel[28]). They thus bear an entirely maternal relationship to the embryo and endosperm, which arise from fusion of nuclei from both parents. Seed development occurs within the embryo sac and several aspects of the structure of the seed are influenced by carpel characteristics. Although variations in such characteristics exist, the structures present are common, and the architecture of the carpel of maize, shown in Figure 4, serves to illustrate the basic structure of all cereals.

When pollen grains fall on the receptive stigma, a pollen tube develops and the two male nuclei in each grain migrate down the style, through the micropyle (the gap where the integuments fail to meet) into the embryo sac. The first male nucleus to reach the female cell fuses with the egg nucleus. Upon fusion, the haploid chromosomes from each

Table III Developmental events in the rye grain (from reference 76)

Embryo development and time after fertilisation	Degeneration phenomenon	Development of embryo-sac and neighbouring tissues
Early segmentation (0–3 days)	Degeneration of synergidae	Multiplication of antipodal cells
Undifferentiated growth (3–6 days)	Degeneration of antipodals	Multiplication of endosperm nuclei Embryo sac passes through first stage of extension
9 days		Growth of nucellar pillar and vascular supply of carpel wall Second phase of extension of embryo sac begins
9–10 days	Nucellar pillar begins to degenerate	Aleurone layer appears (10 days)
First appearance of stem and root apices, and coleoptile primordium (11–17 days)	Degeneration of nucellar pillar and of endosperm in neighbourhood of stem apex, first leaf primodium and scutellary meristem, and of coloerhiza at rot apices	Accumulation of starch in endosperm and protein in aleurone layer
Appearance of first leaf primordium (17 days)		
Appearance of further leaf primordia and secondary roots	Absorption of endosperm in neighbourhood of scutellum	Desiccation of grain an completion of second phase of embryo sac extension (21–40 days)

gametes pair to form the diploid zygote. Division of the zygote leads to the development of the embryo. The second male nucleus fuses with the two polar nuclei to form 'the triple fusion nucleus' (primary endosperm nucleus) which later divides to give rise to the endosperm.

Endosperm

Repeated divisions of the primary endosperm nucleus form the endosperm. There are three types of endosperm development in flowering plants: nuclear (non cellular), cellular and helobial. In nuclear development, the primary nucleus undergoes many nuclear divisions that are not accompanied by wall formation. This results in a coenocyte (a mass of cytoplasm contained within a single continuous plasma membrane, containing many nuclei formed by division of a single original).

The nuclear divisions may be synchronous or asynchronous. As divisions continue, nuclei line the periphery, with the centre occupied by the large central vacuole. A free nuclear condition may persist throughout, or wall formation may take place later, usually occurring centripetally, first initiated at the micropylar region and extending to the chalazal end. In cellular endosperm development the first and subsequent nuclear divisions are followed by cell wall formation. In helobial development the endosperm shows an intermediate condition, in which the primary endosperm nucleus divides into two nuclei with different divisional potencies, one giving rise to a micropylar chamber and the other producing a smaller chalazal chamber. The helobial type of endosperm is exclusive to the monocotyledons and Bhatnaga and Sawhney[73] state that almost all monocotyledons are characterised by helobial endosperm formation. Brown *et al.*[74] state that cereal grains undergo the nuclear type of development with cell walls forming in the immediate vicinity of the developing embryo region earlier than those in the large chalazal region[75].

Starting point

In discussing development of any organism it is convenient to define a datum point from which developmental periods can be defined. Events frequently chosen for most cereals are anthesis and pollination. The latter is the most dependable but it cannot always be clearly identified experimentally, as the precise moment when a successful pollen grain lands on a stigma is difficult to detect, particularly where samples are taken from field grown plants. Time of pollination can only be confidently defined when it is effected manually. Anthesis is the time when anthers first become

Table IV Events in development of embryo and endosperm of the wheat grain (from reference 77)

Stage	Timescale	Development event
I	(0–7 days)	Tissues not yet differentiated and embryo cannot be dissected out
II	(7–14 days)	Embryo can be easily dissected from surrounding tissues and differentiation apparent
III	(14–21 days)	Lateral root primordia initiated
IV	(21–31 days)	Caps found on lateral roots and primary leaves appear
V	(31–50 days)	Fully differentiated. Adhesion to surrounding tissue makes dissection difficult

'Days' refers to the period after anthesis.

visible outside other floral parts. In self pollinating plants such as wheat, it is assumed that anthers shed pollen on to the stigma of the same floret, immediately before they emerge. Anthers emerge first from the basal florets of spikelets just below the centre of the ear, and in most studies ears that anthese on a given day are marked. From marked ears, collected at chosen intervals after anthesis, only basal grains from the central three spikelets on either side of the rachis are selected as it is known that these anthese within a very short period. In maize, male and female flowers are borne on separate inflorescences. The time when receptive stigmas emerge is readily detected and this is a convenient datum point for that cereal.

An ongoing process

The process of development is dynamic and any sequence of events that is defined provides no more than a convenient means of commentating. Similarly, the development of individual components of the grain are often described in isolation although it is recognised that events in one component may influence events in others. One author whose work benefited more than most from a holistic approach is Nutman[76]. His summary of events occurring in the rye grain is given in Table III.

Focusing on development of individual components may nevertheless be useful on occasion, and we include here summaries of events in the major components, embryo and endosperm. Thus, Rogers and Quatrano[77] considered five stages in embryo development in wheat to be significant (Table IV).

There is wide accord as to the phases of endosperm development and Bosnes *et al.*[78] recently defined four similar stages for barley (Table V).

The female gametophyte of cereals at the time of fertilisation

In maize, Diboll and Larson[79] observed a difference in the origin and structure of the wall limiting the central cell in various regions. The wall of the former functional megaspore extends to form the wall of the central cell where it is in contact with the nucellus. Adjacent to the antipodals it is formed by cytokinesis of the megaspore cytoplasm during early gametophyte development. A cell wall and plasma membrane, which are thickest against the nucellus or integuments, surround the central cell. They thin towards the chalazal end of the egg apparatus and in the chalazal region there is no wall between the plasma membrane of the central cell and that of the egg and synergids. The central cell is connected by plasmodesmata with the egg apparatus and antipodals but no such connections exist between the central cell and the adjacent nucellar cells.

Fertilisation and triple fusion

Double fertilisation occurs when two male gametes enter the gametophyte through the micropyle, one of them fusing with the egg cell nucleus to form the zygote, and the second fusing with the two central cell nuclei to form the primary endosperm nucleus or triple fusion nucleus. Morgensen[80] documents fine details of the process in barley. Both male nuclei appear to have no plasma membrane when they enter the egg cell and central cell, respectively. In the case of the zygotic fusion, remnants of the plasma sheath have been identified outside the egg, but no sheath remnants have been seen outside the triple fusion cell.

The cereal endosperm

Coenocyte formation

Percival's observations on wheat[81] revealed that the primary endosperm nucleus divided rapidly following fusion to produce free nuclei, each with five nucleoli, although the egg showed little change. By the time the embryo was 10–15 celled, a single continuous layer of endosperm cells lined

Table V Developmental phases in barley endosperm (from reference 78)

Stage			Timing[a] (days after pollination)	Main events
I.	Syncytial	IA[b]	0	Fertilisation Syncytium[c] formation
		IB	5	Vacuolation of cytoplasm
II.	Cellularisation	IIA	6	Cell wall initiation First cell plate formation
		IIB	8	Cellularisation complete
III.	Differentation	IIIA	14	Vacuolation of aleurone meristem End of starchy endosperm cell divisions
		IIIB	21	End of aleurone cell divisions
IV.	Maturation		40	Accumulation of storage products

Reviewers' notes.
[a] All timings should be regarded only as guides. Details given relate to a single variety of a single species in a single experiment.
[b] A & B are earlier and later phases respectively of each stage.
[c] Syncytium = coenocyte.

the central vacuole. Bennett[82] provided more detail in that the pollen tube penetrated the embryo sac 30 min after pollination. The sperm nuclei reached the egg nucleus and polar nuclei 40–60 min after pollination and the first endosperm mitosis occurred 6–7 h after pollination. The first mitosis in the embryo did not occur until 18–30 h after pollination. Nutman[76] noted that, in the coenocyte lining the embryo sac of rye, nuclei were more abundant on the ventral side, and that the lining layer of protoplasm was also thicker in this region.

Bennett *et al.*[83] examined the very early stages of endosperm development in barley, wheat, rye and triticale. Their interests lay less in morphological relationships than in the rate of cell production. Hence, for the most part, they used preparative techniques that did not preserve tissue integrity. The period they covered was 5 days, from fertilisation to the 5000 + cell stage (estimates of cll numbers in the mature endosperm are cited below). Cellularisation occurred after the 10th cycle (1024 nuclei) up to which divisions were synchronous. The nuclear doubling time increased from 4·5–5·5 h initially, to 8–10 h by the onset of cellularisation 3 days later, and much greater increase followed.

Cellularisation and cell proliferation

Observations of events in several species have confirmed that, just before formation of cell walls, the nuclei are distributed in a thin film of cytoplasm lining the embryo sac and enclosing a central vacuole. With the advent of improved optical techniques, in particular the confocal laser scanning microscope, the role of microtubules has become increasingly clear. Brown *et al.*[74] describe the cellularisation process in barley thus: 'The nuclei become evenly spaced in the cytoplasm in association with the development of radial microtubule systems that emanate equally from interphase nuclei in the syncytium and serve to define domains of cytoplasm around each nucleus. Microtubules of opposing radial systems overlap one another and the cytoplasm remains undivided until ~5 to 6 days after pollination. At this time, numerous microtubules clearly define the nuclear cytoplasmic domains and wall material is deposited in the zones of interaction between opposing arrays' (Fig. 5).

The concept of the 'nuclear cytoplasmic domain', as a unit of cytoplasm capable of being administered by a nucleus, applies to many organisms[84] and it underlies the even spacing of nuclei in the late stage of the endosperm syncytium[85].

In rice, Hoshikawa[86] reported that cellularisation of the lining layer proceeded from the embryo end, resulting in two cellular layers lining the embryo sac. Thereafter all multiplication was by cell divisions, most of which took place in the peripheral cell layers, a process described as centripetal multiplication, which gave rise to 'an orderly arrangement of cells'. By five days after fertilisation the process produced a mass of cells that completely filled the embryo sac. The 14% of divisions that were estimated to occur in the

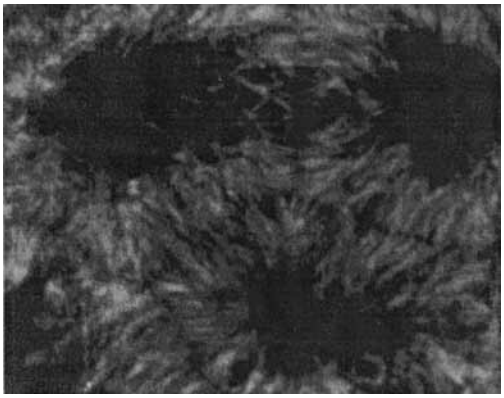

Figure 5 Wall initiation occurring between radial microtubule systems surrounding nuclei of the endosperm syncytium in barley. (Reproduced from reference 74 with permission.)

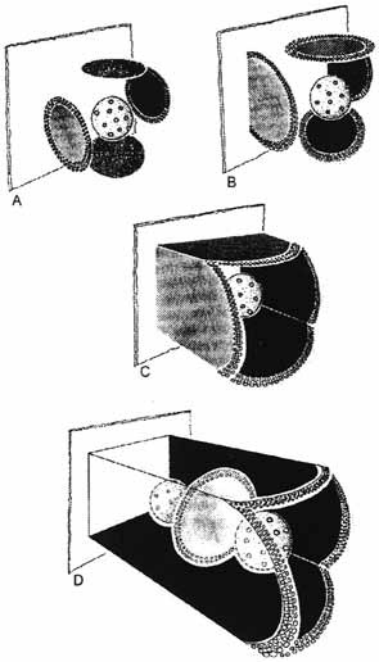

Figure 6 Three-dimensional representation (perspective exaggerated) of the events during the initial formation of walls in endosperm of wheat. Only individual compartments and completed cells are depicted, with closely related stages of wall formation combined into the same diagram. The wall of the central cell is shown at the rear. (A) Pragmoplasts and their first-formed cell plates, grouped around a nucleus, perpendicular to the wall of the central cell. (B) Later stages where cell plates are meeting, and have fused with the wall of the central cell. (C) Further stage where fusion to the central cell is complete and where the anticlinal walls continue to grow freely by their inner margin towards the central vacuole. (D) Stage where a compartment is fully grown and now in the process of periclinal division (top and near side walls not shaded). This division results in the formation of a complete endosperm cell centrifugally and another compartment centripetally. (Reproduced from reference 87 with permission.)

second to fourth layers from the periphery did not have a significant effect on the ordered cellular arrangement. Most divisions took place at night and divisions in both endosperm and embryo were complete after 10 days. It was considered that the rate of division was temperature dependent and that lower temperatures could double the period of division. The early centripetal periclinal divisions in the layer of cells lining the embryo sac occur on the inner faces of the meristematic cells. The daughter cells thus project into a central void and the first of these are moved progressively closer to the centre of the void by subsequent divisions at the periphery. There comes a time, after a few divisions, when the first daughter cells of the cellular meristem on one side meet with others originating from meristematic cells on the opposite side. Considerable effort has been applied to the manner in which cells that meet relate to those that they encounter.

Fineran *et al.*[87] described the process of wall formation in wheat endosperm in some detail (Fig. 6). Anticlinal cells first appeared in the crease region and progressed all round. The process was typical of normal cytokinesis, with the short-lived appearance of a phragmoplast, with vesicles fusing to form a cell plate that expanded to the full dimensions of the cell face. Such development of anticlinal walls without formation of periclinal walls gave rise to 'open cylinders' or alveoli[88,89] The cylinders remained open on the inner face although normal cytokinesis gave rise to walls on the outer face. Periclinal walls on the innermost cells also appeared first in the crease region, with walls of several adjacent cells occurring simultaneously. The 'closing phase' proceeding into the checks consisted of the movement of nuclei from the leading edge into the centre of the alveoli, and formation of partition periclinal walls from adoposed anticlinal wall margins.

Brown *et al.*[74] describe the changes in the innermost layer of alveoli (they are not 'cells' because they lack inner perclinal walls) thus: 'Concurrent with the initiation of walls in boundaries of non-cellular domains at 5–6 DAP, the cytoplasm becomes vacuolate and non-cellular domains begin to bulge into the central vacuole. As the nucleate columns of cytoplasm grow inward, the interphase

microtubules become rearranged. Microtubules envelope the elongating nuclei and flare from both ends, forming a plate at the base nearest the nucellus and a crown of microtubules in a thin layer of cytoplasm adjacent to the central vacuole. At 6–7 DAP the cytoplasm becomes shaped into configurations resembling trees. By 7 DAP the interaction of opposing microtubules emanating from adjacent nuclei gives rise to phragmoplasts that form adventitiously in the cytoplasm.'

The number of cells formed by the peripheral divisions of rice may have varied, as different numbers of cell layers resulted in different regions of the endosperm and 19–20 layers were counted on the dorsal side while up to 16 were recorded on the lateral and ventral sides. The number of peripheral cells counted in a transverse section was 200, while 150 were counted along the longitudinal diameter.

Some patterns observed have indicated that cellular division had occurred in the layer just within the outermost layer, confirming the conclusion of Hoshikawa[86] and others that a minority of mitotic divisions take place in layers other than the peripheral cells. Bosnes and Olsen[90] place far less emphasis on the importance of cell divisions in the outermost cellular layer or layers, and indeed suggest uniformity of mitotic activity within the endosperm. Further, the implication of this contention is a separate origin of the different classes of starchy endosperm cells. Support for this view is perceived in work with mutants, in which endosperm development was incomplete. Thus failure of development on one flank and on the central dorsal side of grains was interpreted as indicating separate and distinct origins for the regions, which could be traced back to descendance from right and left daughter nuclei resulting from the first endosperm nuclear division. This is consistent with observations of McClintock[91] who showed that, although all cells in the endosperm descend from the single primary endosperm nucleus, they are capable of expressing different genes. Apart from differentiating into either aleurone or starchy endosperm cells, a clone of cells descended from a single 'parent' may display a characteristic feature common to the clone but different from cells descended from other 'parents'. Examples of clonal differences were the ability or not to synthesise anthocyanin pigments among aleurone clones and waxy rather than wild type starch production in starchy endosperm cells. By introducing such markers by which clones can be visually recognised, the origin of cells in different parts of the endosperm were determined. A difference between endosperm cells in the crease region and elsewhere was demonstrated by Doan *et al.*[92], who found that a cDNA hybridised only with endosperm in the crease region. Hoshikawa[93] discovered that most of the meristematic activity in the endosperm occurred at night and this possibly explains why other authors have witnessed only a fraction of the number of mitoses that must occur in transforming the 5000 cells of the coenocyte into possibly 300 000 at maturity. Hoshikawa's unique quantitative assessment that 86% of all divisions occur in the outermost layer provides weighty support for the peripheral meristem interpretation. However, over a long period, alternative interpretations have been well supported. The older literature has been reviewed[94], but it is appropriate to review the observations that endorse the view thhat we still maintain. Many studies have drawn attention to the ordered array of cells that radiate from the periphery to the centre of the endosperm, or in the case of caryopses with a crease, to the centre of the flanks (Fig. 7). This in itself is suggestive of a common origin but the fact that the size of cells increases towards the centre within each file is compatible with the first formed cells (those at the centre) having had longer to accumulate storage products, particularly starch, and hence to expand most. In wheat, the size of lenticular starch granules is relatively uniform within a cell but granule size declines systematically among cells from the centre to the periphery.

A coincident decline in the numerical complement of lenticular granules was discovered by Hughes and Briarty[94], who found that the decline progressed geometrically and was hence compatible with a sequence of peripheral divisions in which each daughter cell received half the number of amyloplasts or proplastids.

An estimate of 180 000 was made for the total number of cells present in the endosperm of rice (20–100 more cells have been counted on the longitudinal diameter of long-grain varieties than on that of short-grain varieties)[93]. Estimates in barley include 70 000 starchy endosperm cells[78] and 100 000 aleurone cells[95]. Cochrane and Duffus[96] found that maximum starchy endosperm cell number was achieved by 14 DPA but in the triple layered aleurone tissue the maximum number was not present until 21 DPA. Brunori *et al.*[97] found 130 000 to 180 000 cells in wheat endosperm (aleurone cells exluded), with all grains

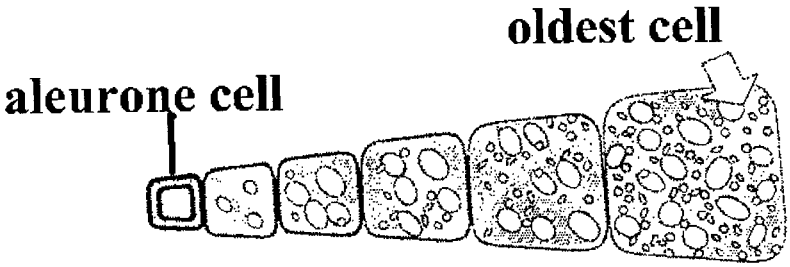

Figure 7 Diagram of a column of cells generated from a single meristematic cell, which subsequently differentiated into an aleurone cell.

weighing over 45 mg having over 150 000. Evers[98] cited between 32 000 and 300 000 for the same species. Reddy and Daynard[99] estimated cell numbers in a variety of maize types and found significant differences among them. In a flint-dent adapted hybrid, the largest grains, located near the base of the cob, were estimated to have 176 000 cells while smaller grains from the tip of the cob had only half to one third that number. The smallest grains examined from a decorative ultra-small popcorn averaged 35 000 cells over two years.

One means of estimating cell number is division of total endosperm DNA by the known amount of DNA per cell. Because some endosperm cells are polyploid this can be misleading unless allowance is made for this. Such polyploidy may be due either to fusion of adjacent nuclei before wall formation[100] or to several other processes such as endomitosis, spontaneous C-mitosis, endoreduplication, fusion of adjacent spindles at anaphase, and persistent bridges[101]. Chojecki *et al.*[102] found that approximately 10% of cells were 3*C*, 55% were 6*C*, 25% were 12*C* and 10% were 24*C*. Before 12 days after anthesis only 3*C* and 6*C* were present while 12*C* cells arose at 12 days and 24*C* cells at 15 days. Based on these assumptions, a large grained wheat variety (cv. Spica) had 282 000 and a smaller wheat variety (cv. Chinese Spring) had 240 000 cells at maturity. Ploidy levels were also similar for the two varieties. Brunori *et al.*[97] found a weak correlation between grain mass and a function of % ploidy in the full population studied, but a weaker relationship with cell number. In some genotypes, grains over 50 mg were associated with high numbers of polyploid cells but low cell numbers.

Polyploid aberrant nuclei were significant features in triticale endosperm in some of the early intergeneric hybrids. Premature production of hydrolytic enzymes by aleurone cells that were thus affected may have been a consequence. Failure of cells to divide after DNA synthesis was attributed to lack of synchrony in chromosome doubling in the wheat and rye genomes[82].

It has been reported for several cereals that most nuclei with a DNA complement greater than the normal 6C were located in the neck of endosperm close to the proembryo[103]. Bennett[82] noted in triticale (cv. Rosner) that giant polyploid nuclei, occurring early in coenocyte development, when numbers of nuclei are low, could be lethal to the tissue, while at later stages, when numbers were higher, the death of only the cell concerned resulted. Another feature of endosperm with nuclei containing between 2 and 16 fold the normal complement of DNA was the tendency for cell walls to form earlier.

Differentiation

Hoshikawa's[86] account of cellular divisions in rice endosperm noted that, when peripheral cell divisions ceased, the walls of the outermost layer thickened and, in stained sections, granular cell contents and a large distinct nucleus became visible. In other words, the formerly meristematic cells differentiated into an aleurone layer. The differentiation extended to three to six layers in the area adjacent to the vascular tissue in the pericarp and the process occurred prematurely. Plasmodesmata were detected in aleurone cell walls and those in the multi-layered ventral region displayed features associated with transfer cells.

Fruit and seed coats

Nutman[76] examined median longitudinal sections of rye grains and recorded lengthwise extension of the embryo sac through extension growth and

cell division in the surrounding tissues. The extension growth occurred at the distal end of the carpel and there is evidence from the shape of mature pericarp epidermal cells that this is also true of wheat, as cells are isodiametric at the embryo end and elongated parallel to the grain's long axis elsewhere (cells at the distal tip, bearing trichomes, are also isodiametric). The elongation occurred in two phases, with a discontinuity at 10 days post anthesis, corresponding with the time of most rapid expansion of embryo and endosperm. An interesting comparison between spring and winter types of the same rye strain showed that although both achieved the same final length, the spring type grew more rapidly. In the early stages of most rapid extension of the embryo sac, distal parts of the enclosing cell layers elongate by extension growth. Later, when growth is slower, increase in covering layers is by intercalary meristematic zones in the basal halves.

Nucellus and integuments

Failure of the outer integument to grow for most cereal species has been reported, resulting in the remaining seed coat having originated in the inner integument. The development of cuticles on the outer faces of both nucellar epidermis and testa of wheat have been described by Morrison[104].

Whereas most nucellar cells degenerate, those in the ventral region expand and multiply. Those in the antipodal area expand but other cells produce the nucellar pillar, initially by expansion followed by meristematic activity. The vascular bundle in the crease also extends by cell division.

Pericarp

Van Lammeren[72] described three zones in maize pericarp at three days after pollination. The endocarp comprised about three layers of thin walled cells that elongated at right angles to the proximal–distal axis. The mesocarp consisted of larger, isodiametric, loosely arranged cells and the exocarp of cylindrical cells with little intercellular space. Features common to all pericarp cells were little cytoplasm and a large central vacuole. With grain expansion due particularly to endosperm growth, exocarp cells increased in length, mesocarp cells elongated and adopted a curled configuration and endocarp cells separated into fibrous sheathes with large spaces between. Further expansion led to crushing of all cells except those in the exocarp, the walls of which had become thickened. All pericarp cells were by this time devoid of cytoplasm. A study by Morrison[105] chronicles the detailed ultrastructural changes in the chlorophyll containing cells that become the tube cells (inner epidermis) and cross cells of wheat.

Vascular tissues

Simple vascular bundles are embedded in the pericarp of developing grains of wheat, rice and other cereals that possess anatropous ovules. Vascular tissue in the chalazal region delivers nutrients to the endosperm and other vascular tissues, which were concerned with transport to floral parts such as anthers and style persist. The path from the chalazal vascular strand to the developing filial tissues (embryo and endosperm) has been studied in several grasses and legumes and it is now well established that these are isolated from maternal tissues, with no plasmodesmatal connections[106]. During the period of rapid grain growth, symplasmic continuity exists between the ovular vascular tissue and the nucellus only through a chalazal zone, which at maturity becomes identifiable in wheat as the pigment strand[107]. The pigmentation arises as a result of accumulation of adcrusting substances that are coloured (in red wheats). A similar tissue exists in rice but it does not become pigmented. Lying between the vascular tissue/pigment strand and the endosperm in both wheat and rice is a persistent pillar of nucellar tissue via which nutrients pass inward to the filial tissues. A notable feature present in wheat but absent or less obvious in rice is a large discontinuity described as the endosperm cavity. A feature that is common to both species however is the modified aleuronic tissue in the region.

Hoshikawa[93] describes the vascular bundle as like the pipes of a pipe organ, each terminating at different heights so that substances translocated upward through the pipes are discharged to the endosperm from the whole 'dorsal' side of the grain. Before differentiation of the aleurone layer, nutrients enter via the nucellar epidermis while later they are restricted to passage through the nucellar projection.

Relationships among events in the embryo sac

Nutman's record of some of the coincident events occurring in rye grain development[76] is reproduced earlier in this review (Table III) as a summary of development, but without drawing attention to any possibility of relationships among those events.

Nutman himself, however, used the table to illustrate the possible causal relationships of one to the other and indeed he observed physical relationships that endorsed these possibilities. The correlation of these processes may be related to the nutritional value of the resorbed tissues, or to the liberation of growth-promoting substances that stimulate the development of cells nearby. He drew particular attention to the earlier events. As described above, cell division in the coenocytic endosperm is synchronous until the tenth division, after which cellularisation occurs and divisions become asynchronous. Divisions are not random, however, and patterns have been discerned. At the time of fertilisation five antipodal cells were present. Hoshikawa[86] noted that divisions in the endosperm mother cell started in close proximity to the antipodal cells which began to degenerate after fertilisation and had disappeared five days later. Nutman's observation of antipodal cells in rye[76] revealed that, at the time of fertilisation, cells became vacuolate. They extended into the embryo sac as loose tissue attached at the chalazal end. After fertilisation, cells and their nuclei increased strikingly in size before undergoing rapid degeneration. Cell sizes were 10 μm before fertilisation, 36 μm at fertilisation and 54 μm just before disintegration. The number of antipodal cells varied, with 6–18 having been observed and some cells having up to three nuclei. Endoreduplication has been observed in antipodal cells of wheat with ploidy levels of $1C$ to $256C$ having been recorded[82].

Nutman[76] examined 20 sections of rye grains in which the endosperm was at the free nuclear stage, with the syncytium forming a lining layer of the embryo sac. Only two sections showed mitotic activity, but both were in the same stage of nuclear division. Earliest stages of mitosis were seen on the ventral wall, closest to the antipodals, with mitotic phases (metaphase to anaphase) progressing with increasing distance from that point. The progression was radial over 360 and not restricted to a single plane. It suggested to Nutman that dividing nuclei moved from a focus in an orbital flow of cytoplasm. While there was no direct evidence for the influence of the antipodals, the pattern was consistent with the presence of an agency, such as a hormone capable of initiating division. An analogous situation was reported in lactifers of *Euphorbia marginata* Pursh, where mitotic waves were observed[108] Mahlberg and Sabharwal attributed this phenomenon to diffusion of a substance that stimulates mitotic activity along an axis[108]. Presence of a similar substance surrounding developing endosperm may be the agent by which meristematic activity is maintained during the cell proliferation phase.

Nutman strengthened his argument concerning the influence of a degenerating component on the development of a developing one with evidence for similar relationships in other plants and by describing concurrent events in other tissues close to the antipodals[76]. He noted that during the enlargement of the antipodals, expansion of the embryo sac proceeded at a rate of 13% per day but during antipodal degeneration the rate increased by 113% per day. No further growth occurred after the disappearance of all residues of the antipodals. Further support comes from the detection of growth-promoting substances produced in autolysing tissues[109].

Subsequent events consisted mainly of rapid nuclear division in the nucellus, giving rise to the nucellar pillar and growth of the vascular strand. Nutman conjectured that hormones responsible for promoting cell expansion and multiplication may have been translocated via the vascular tissue from tissues dying elsewhere in the plant. What is described as the almost complete digestion of the nucellar pillar was observed to coincide with the initiation of differentiation of the aleurone layer.

A further possible influence on one tissue by an adjacent one is the formation of the crease. Evers[24] showed that the number of cells between the crease aleurone and the dorsal aleurone was approximately half that between diametrically opposite aleurone layers in the lateral parts of the grain. He interpreted this as a result of failure of peripheral endosperm cells, adjacent to the nucellar projection, to divide, while those elsewhere continued to do so. This is consistent with earlier differentiation to a double-layer of aleurone cells in the crease region. Premature differentiation into cells of a somewhat distorted and thick walled aleurone has been noted in equivalent endosperm regions of other cereals, even some that do not have a crease (e.g. rice[86]).

Wheeler[110] showed that a decline in cytokinin corresponded with the end of mitosis in wheat, but we are not aware of any recent work that has sought to confirm the presence of growth promoting substances in areas of growing grains where meristematic activity is maintained or terminated as proposed by Nutman[76]. Nor has any alternative mechanism been proposed for locally

initiating, sustaining and terminating such activities.

The apparent early death of aleurone cells in the crease region, together with their tendency to form cell walls prematurely, are characteristics of polyploid cells (see section on Endosperm cell numbers and ploidy, above). This possibility is also compatible with the discovery by Brunori *et al.*[97] of a positive relationship between grain size and number of polyploid cells and evidence for a similar relationship between grain size and number of crease endosperm cells[70]. However, no information exists as to a possible concentration of polyploid cells in this region.

Cereal endosperm as a model of morphogenesis

Authors have drawn attention to the value of cereal endosperm as a model system for morphogenesis studies. Its ordered structural development, with fundamental similarities extending over many species, but with significant variations related to developmental behaviour and final condition, offer great opportunities for relating cause and effect.

The concept of a solid mass comprising radiating columns of cells that are largest in the centre is not sustainable unless the smaller cells increase in number towards the periphery. Anticlinal divisions are thus a necessary component of the development process. Evidence of such divisions has been cited and it is reasonable to assume that they occur along two perpendicular axes. However, little is known about the sequence of the respective anticlinal divisions one to another and to the centripetal periclinal divisions. Considerable progress has been made towards creating satisfactory algorithms appropriate to tissues comprising a single cell layer. The concept of a deterministic system, whereby the plane of one cellular division determines the next, has been shown frequently to apply in such a two-dimensional tissue. A primitive attempt[98] to devise a possible morphogenic model for wheat endosperm assumed, without evidence, a deterministic sequence of two perpendicular periclinal divisions and one anticlinal division in the meristematic aleurone. It also took account of the fact that the number of modified aleurone cells increases during the cell division phase. It therefore included the concept of progressive premature differentiation in the expanding crease region.

In this context, the work of Van Lammeren[72] is relevant. His observations were concerned less with control mechanisms and more with mechanical/cellular mechanisms by which the morphology of the grain and that of its components are directed. In examining the orientation of microtubules he distinguished those that form the spindle from those that occur throughout the cytoplasm and a third group concentrated in a peripheral zone of the cytoplasm. He investigated, in maize grains, the agents that induce polarity on the embryonic axis and considered factors that influence meristematic activity leading to development of major organs (shoot and root). He concluded: 'Embryo development is determined or influenced by the initiation and maintenance of polarity. The shape of the embryo is further influenced by the formation and activity of meristems and the directed cell elongation in the various organs.'

In spite of the availability of enormously improved facilities, microscopical methods still allow only limited opportunities for dynamic studies. Each destructive observation is thus a mere snapshot and the relationships within a sequence of divisions in a meristematic tissue are difficult to determine and the orientation of the spindle in a newly formed cell may owe much to that of its mother cell. The concepts concerning the influence exerted by one caryopsis component upon another first presented by Nutman[76] have been reiterated, rediscovered and pursued but, as yet, no comprehensive thesis has emerged. The differing behaviour of endosperm cells close to those cells that persist in a functional condition, from that of endosperm cells elsewhere remains a particularly intriguing aspect.

Concluding remarks

The scope of the subject of this review is enormous and selection of the aspects to be covered is clearly subjective. We have concentrated on the broad picture, often to the exclusion of the individual components of grain tissues. Thus, scant attention has been paid to interesting and important aspects such as development and detailed comparisons, among the cereal species, of storage compounds such as starches and proteins. Similarly, our concentration on earlier post-fertilisation caryopsis development has led to omission of later events and mechanisms such as water loss and programmed cell death – fields on which further research is undoubtedly merited. Never-

theless, it is hoped that this review will prove useful when used in conjunction with knowledge that is emerging and with the many reviews and original publications that exist on some of the topics that we have neglected.

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