Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.)

S KRISHNAN† and P DAYANANDAN*

*Department of Botany, Goa University, Goa 403 206, India
†Department of Botany, Madras Christian College, Tambaram, Chennai 600 059, India

Corresponding author (Fax, 91-832-2451184; Email, skrish8@yahoo.com)

The endosperm and embryo that constitute the filial tissues of rice caryopsis are isolated from the maternal tissues by the absence of any symplastic continuity. Nutrients are transported to the endosperm through a single ovular vascular trace present on the ventral side of the ovary. Initially solute enters through the chalaza into the nucellar projection and then into the endosperm. At later stages transport occurs through the nucellar epidermis, centripetally towards the endosperm. The cell walls of the nucellar epidermis are provided with rib-like thickenings. A comparison of grain-filling in C<sub>3</sub> and C<sub>4</sub> cereals suggests that rice has structural features allied to C<sub>3</sub> cereals, such as wheat, but with significant differences.

1. Introduction

The development and structure of rice grain are fairly well understood (Santos 1933; Juliano and Aldama 1937). Recently Jones and Rost (1989) and Bechtel and Pomeranz (1977, 1978a,b) investigated the ultrastructure of developing rice embryo and mature rice grain. The possible route of entry of assimilates into the developing rice caryopsis was studied by Hoshikawa (1984) and Oparka and Gates (1981,a,b, 1982). We have previously reported on the structure of rice caryopsis in relation to yield (Ebenezer et al 1990, 2001), and on the histochemical localization of major storage components (Krishnan et al 2001). We report here our findings on the transport of assimilates and major nutrients in developing grains and the storage components in mature caryopsis of rice.

2. Methods

2.1 Plant material

An indica rice, *Oryza sativa* cv. IR50, was the central focus of study. However, several other cultivars and species obtained from the International Rice Research Institute (IRRI), Philippines, and local sources were examined to compare and confirm the observations made on IR50. These other rices include: cv. Ponni, IR20, and ADT36 (from Tamil Nadu Agricultural University, Coimbatore), J13 (from J-Farm, Kelambakkam, Tamil Nadu) and *Oryza alta* Swallen, *O. australiensis* Domin, *O. barthii* A Chev, *O. brachyantha* A Chev & Roehr, *O. eichingeri* A Peter, *O. glaberrima* Steud, *O. grandiglumis* Prodh, *O. granulata* Nees. et Arn, *O. latifolia* Desv, *O. longiglumis* Jansen, *O. longistaminata* A Chev et Roehr, *O. minuta* J S ex C B Presl., *O. nivara* Sharma & Shastry, *O. officinalis* Wall ex Watt, *O. punctata* Kotschy, *O. ridleyi* Hook f and *O. rufipogon* Griff (from IRRI). Unless otherwise specified data and figures refer to IR50.

2.2 Microscopy

Free-hand sections, wax and Spurr plastic-embedded thin sections were stained with a variety of bright-field dyes and fluorochromes using standard histochemical procedures (Pearse 1972, 1980; Fulcher 1982; Harris and Oparka...
Movement of phloem-specific fluorochrome 5 (6)-carboxyfluorescein through the vascular trace and nucellar epidermis was studied by examining sections of the caryopsis at different times after placing the cut end of a pedicel in 0.01% dye solution. Specimens were examined and photographed with a Nikon Microphot-FXA provided with bright-field, dark-field, phase-contrast, Nomarski-DIC, polarized light and fluorescence modes of examinations.

3. Results

The term ‘caryopsis’ refers to the fruit of rice which at maturity consists of a thin and dry pericarp, a bulky endosperm with an outer layer of aleurone cells, and an embryo. The developing caryopsis of rice is hermetically enclosed within the space provided by two tightly clasp- ing fertile glumes of the spikelet, the palea and lemma. A rice spikelet has other associated structures namely, rachilla, sterile lemmas, and rudimentary glumes. An abscission layer occurs immediately below the sterile lemmas. The terms ‘grain’ and ‘paddy’ should refer to this collective unit, and the term caryopsis should be restricted to the fruit of rice. One of the important but poorly understood aspects of grain-filling in rice is the relationship between the caryopsis and the sterile outer coverings, particularly the palea and lemma. In addition to offering protection against insects and fungi, the palea and lemma may contribute assimilates to the developing caryopsis, regulate water balance during grain-filling and impose a limit on the size of the caryopsis (Cochrane and Duffus 1979).

3.1 Development of ovary and pericarp

The changes in size and shape of the rice ovary from the time of flower opening (anthesis) to 30 days after fertilization (DAF) are summarised in figure 1. Immediately after fertilization rapid changes occur in the tissues of caryopsis: i.e. pericarp, vasculature, integuments, nucellus, endosperm and embryo. The structure of the ovary and caryopsis at three successive stages of development, as seen in transverse sections, is illustrated in figures 2–4. The ovary wall at the time of anthesis consists of about 7–10 layers of cells (figure 2). Embedded within the ovary wall are vascular tissues that supply water and nutrients to the stigma and the ovule. The outer epidermis of the ovary is covered by a thin-layer of cuticle. The inner epidermis consists of smaller cells that later develop into tube-cells. Two or three layers of subepidermal cells of the inner epidermis develop into cross-cells (figures 3, 6a). During the first six DAF the caryopsis rapidly elongates and reaches a maximum length of about 8 mm. The inner epidermal cells separate from each other, elongate longitudinally (parallel to the long axis of the caryopsis), and form an extensive network of tube-cells that enclose the developing endosperm. The functional significance of tube-cells is not known. They might offer mechanical support or may be involved in short distance transport of...