CELL-WALL POLYSACCHARIDES OF DEVELOPING RAPESEED

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INTRODUCTION

The development of rapeseed has been defined in terms of morphological, anatomical and chemical changes occurring from shortly after anthesis through to seed maturity (Norton *et al*, 1978). some of the ultrastructural changes in the cotyledonary cells of the embryo particularly during oil and protein deposition have also been investigated. On the basis of these studies, rapeseed embryogenesis has been divided into three stages: Stage 1 in which embryonic cell division is accomplished; Stage 2 when cell expansion and deposition of storage material and protein occurs; Stage 3 which involved the dehydration and maturation of the seed. Compositional studies on developing rapeseed have been restricted almost entirely to storage carbohydrate, soluble sugars, protein and oil contents and compositions (Norton and Harris, 1975). Little attention appears to have been paid to the cell wall polysaccharides in developing rapeseed.

Cell wall polysaccharides accounted for between 22 and 29% of the dry matter of dehulled-and defatted-rapeseed meals of *Brassica napus* (Theander and Aman, 1977) and *B.campestris* (Siddiqui and Wood, 1977) respectively. The major cell wall polysaccharide components of *B. campestris* were found to be pectin (14.5%), cellulose (7%), amyloid including fucoamyloid (4.5%), arabinogalactan (1%) and arabinan (2%) (Siddiqui and Wood, 1977).

The purpose of this investigation was to follow the changes in cell wall components throughout seed development in relation to other compositional and dry matter changes.

MATERIALS AND METHODS

Seeds of winter rapeseed (*B. napus ssp oleifera cv Ariana*) were obtained from commercial crops grown on the University of Nottingham farms. Pods were harvested at regular intervals usually weekly from shortly after anthesis to maturity. After removal from the pods the seeds were quickly frozen and freeze dried. Seed development was monitored as individual seed dry weight, oil content and oil composition. Using this information, seed development was related to the more detailed compositional and ultrastructural studies carried out previously. Lipid was determined as outlined previously (Norton and Harris, 1975). Seeds for cell wall analysis were defatted by grinding and extracting three times in petroleum ether. A cell wall fraction was obtained by removal of starch and protein enzymatically (Englyst and Cummings, 1984; Englyst *et al*, 1982). Total cell wall and non-cellulosic polysaccharides were determined following the hydrolysis of the cell wall fraction with 12 M and IMH_2SO_4 respectively. Neutral sugars were estimated by quantitative G.C. as the corresponding alditol acetates following reduction and acetylation. Uronic acids were determined by the method of Scott (1979).

RESULTS AND DISCUSSION

Seed dry matter and oil accumulation (Fig. 1) was similar to that described previously (Norton and Harris, 1975). It is evident, however, from the seed dry weight and oil content that although the first seed harvest was taken 10 days after anthesis, phase

1 of seed development (cell division) was or was almost completed and phase 2 (cell enlargement and rapid dry matter accumulation) had commenced. At this stage the embryo would have contributed little to the total seed dry matter, which would have consisted mainly of integuments and endosperm tissue. In phase 2 there was a rapid increase in both seed and embryo dry weight which was associated with the deposition of oil and protein reserves. No cell division occurred during this period but cell expansion was rapid as was reflected by a 10 fold plus increase in cell volume. Phase 3, seed maturation, occurred with little further dry matter accumulation.

This preliminary investigation on cell wall development was carried out on intact ovules because of the small size and physical impossibility of excising sufficient embryos, particularly in phases 1 and 2 for this type of analysis. The analysis undertaken was also determined by the availability of the experimental material. Clearly insufficient material was available to undertake the fractionation of rapeseed cell wall polysaccharides as carried out on mature dehulled seed (Siddiqui and Wood, 1977; Theander and Aman, 1978).

Despite these inadequacies certain features of the study are worthy of comment. In contrast with the accumulation of dry matter associated with protein and oil deposition, cell wall polysaccharide synthesis was particularly rapid during the later stages of phase 2 and early phase 3 (Fig. 2).

The individual polysaccharide components of the cell wall were not synthesised in synchrony. Galacturonic acid and presumably other constituent sugars of the pectin fraction [(arabinose, galactose, xylose and fucose (not assayed in this study)] were deposited early in embryo (ovule) development and thereafter remained constant to maturity. Glucose associated mainly with the cellulose component of the cell wall, was synthesised at a rapid and uniform rate throughout development. Arabinose was present in relatively small amounts in early seed development and did not increase until the rapid phase of storage oil and protein deposition. The synthesis of cell wall polysaccharides containing arabinose was particularly rapid over this period and at maturity this pentose was the major cell wall constituent. Xylose was present in very low amounts in the cell wall polysaccharides during early seed development and only appeared in appreciable amounts in the later stages of seed development. At maturity xylose and galactose were present in equal amounts in the cell wall polysaccharides. Galactose, galacturonic acid and glucose were the major sugar components of the cell wall polysaccharides in early seed development. Galactose declined at the time of rapid dry matter accumulation in the seed but then increased at a rate commensurate with that of xylose. Overall it would appear that cell wall polysaccharides containing arabinose and to a lesser extent xylose were synthesised mainly during the later stages of seed development.

Cell wall development in rapeseed tissues in which cell expansion and lipid and protein deposition is occurring follows a specific pattern. Pectins associated with the middle lamella of the cell wall were synthesised in their entirety after the completion of cell division and before appreciable cell expansion took place. This observation confirms earlier ultrastructural studies on the embryo after the completion of cell division and at the onset of cell expansion (Norton *et al*, 1978). As cell expansion proceeded the cell wall became more distinguishable in electron micrographs due to the deposition of cellulose and other polysaccharide components of the primary cell wall. The synthesis of these materials was essential for the cell walls to accommodate the requirements of cell expansion. Non-cellulosic and non-pectin polysaccharides were deposited later in seed development and at maturity these accounted for the majority of the cell wall polysaccharides.

Although no polysaccharide fractionation was carried out in this investigation limited conclusions may be made with respect to certain polysaccharide types from the

preliminary analytical data presented. Pectins and associated arabinogalactans were synthesised early in embryogenesis. Cellulose appeared to be synthesised throughout seed development. Amyloid, fucoamyloid, arabinan and acidic xylan would be synthesised during the later stages of embryo cell expansion and maturation.

CONCLUSIONS

Cell wall polysaccharide synthesis was slow in the cell division and early cell expansion phases of seed development but was extensive during the rapid accumulation of dry matter mainly as protein and oil. Individual cell wall constituents were not synthesised in synchrony. Pectins were deposited in early seed development. Cellulose increased at a constant rate throughout seed development. Polysaccharides containing arabinose and to a lesser extent xylose and galactose were synthesised throughout development but especially in the later stages of development. The sequence of polysaccharide synthesis and deposition appeared to be determined by the specific structural requirements of the cells at the respective developmental stages.

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Fig. 1 Pattern of seed development of winter rapeseed (*B. napus*)

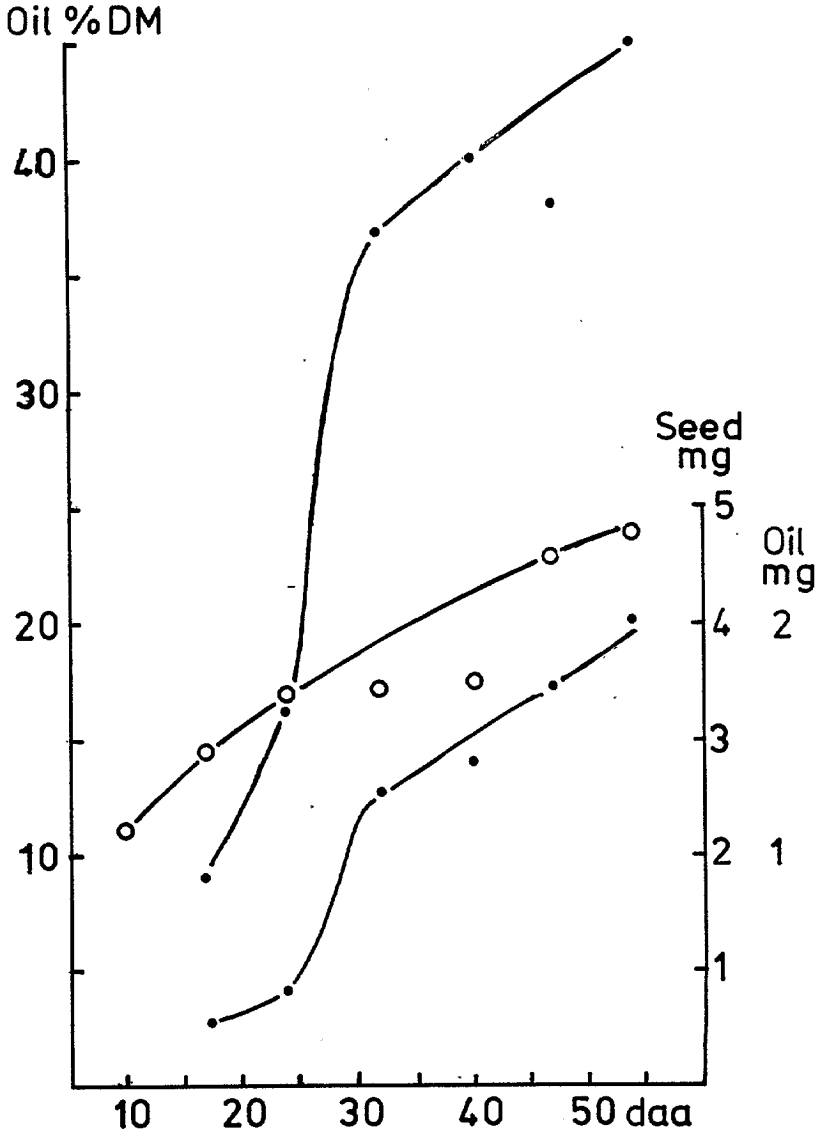

Fig. 2 Accumulation of cell wall carbohydrates in rapeseed during development