BIOCHEMICAL AND DEVELOPMENTAL PROCESSES ASSOCIATED WITH POD SHATTER IN OILSEED RAPE

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ABSTRACT

Cells in the dehiscence zone (DZ) of oilseed rape pods cv. Fido, separated at the middle lamella eight weeks after anthesis. This was preceded by reductions in the amounts of auxin and ethylene. Seven genes encoding for β-1,4-glucanase and six for endopolygalacturonase (endo-PG) have been amplified using a nested polymerase chain reaction (PCR) approach. One isoform of each enzyme was localised in the DZ.

INTRODUCTION

There is insufficient variation in the structure of the siliqua of present cultivars of oilseed rape (*Brassica napus* L.) in order to improve resistance to pod shatter by classical breeding procedures. The overall aim of the work presented in this paper was to improve knowledge of the developmental and biochemical events which result in cell separation in the DZ, thereby providing information which could lead to the eventual production of transformed plants with reduced shatter susceptibility.

The DZ is composed of a layer of two or three parenchymatous cells situated between lignified cells of the carpel edge and replum. After separation, the cells at the fracture surfaces are smooth and whole, and separation appears to depend upon dissolution of the middle lamella. The role of hormones in abscission has been the subject of much research and Sexton *et al.* (1985) have suggested that regulation of the process is under multifactorial control. This involves reduction of auxin status prior to lowering of ethylene and subsequent acceleration of abscission involving abscisic acid (ABA). Meakin and Roberts (1990) reported that ethylene produced by intact pods rose to a climacteric during maturation and preceded a rise in cellulase (β-1,4-glucanase) activity in the DZ. Cellulase is involved in the degradation of cellulose, and possibly xyloglucans in cell walls but does not alone account for the breakdown of the pectinaceous substrate of the middle lamella. Changes in the levels of production of ethylene, the auxin indole-3-acetic acid (IAA) and ABA, and of cellulose and pectin-degrading enzymes in the DZ, pod wall (PW)

and seeds of oilseed rape during senescence are reported in this paper.

RESULTS AND DISCUSSION

Anatomical analysis

Pods were removed at weekly intervals after anthesis from plants of the spring oilseed rape cv. Fido growing in a glasshouse. Tissues were fixed in formaldehyde/ gluteraldehyde mixtures for light or electron microscopy. Cells of the DZ were fully vacuolated two weeks after anthesis. The cytoplasm remained clear and contained active organelles for a further two-three weeks, after which, deterioration of cell contents was clearly visible. Fragmentation of cytoplasm took place six or seven weeks after anthesis. The structure of the cell wall began to change about a week later. The primary cell wall became less dense and appeared to be thinner by the time separation along the middle lamella started eight weeks after anthesis.

Hormone status during cell wall degradation and separation

Whole pods for ethylene analysis were enclosed in sealed tubes and incubated at 25°C. A 1.0 cm^3 sample of gas was removed after one hour and injected into a Pye Unicam 204 gas chromatograph incorporating a hydrogen flame ionisation detector and fitted with an alumina-packed column through which nitrogen flowed as a carrier gas. Increased ethylene production prior to cell separation, as reported by Meakin and Roberts (1990), was found to take place in our material between four and five weeks after anthesis. Production at this peak level was maintained for ten days before falling rapidly.

Pods were dissected into DZ, PW and seeds. Separated tissues were frozen in liqid N_2 and stored at -80°C. This was used for the determination of ABA, and free and conjugated forms of IAA by methods described by Bialek and Cohen (1989) and Prinsen *et al.* (1991) respectively. The immediate precursor of ethylene, aminocyclopropane carboxylic acid (ACC) was analysed by the method of Chauvaux *et al.* (1993). In all tissues, peak concentrations of ACC coincided with the ethylene peak. However, the amount of conjugated ACC increased after ethylene production declined. Values in the DZ and the PW were much greater than those in the seeds. Concentrations of free and conjugated forms of IAA were greatest in the seeds and lowest in the PW. In the DZ, values for free and bound forms increased between three and six weeks after anthesis, slightly anticipating the rise in ethylene but declined from six weeks onwards, as ethylene levels also fell. ABA increased in pericarp tissues seven weeks after anthesis.

Biochemical and molecular biological investigations of enzymes

Frozen tissue was ground in liquid nitrogen followed by homogenisation in 10 mM MOPS (5ml per gram fresh weight (FW), pH 7) with sand and 1 mM PMSF. After centrifugation, 15 mins at 12000 x g, the pellet was washed in a large excess of water, re-centrifuged and stirred for 3 hours at 4°C with 4M LiCl, 5ml per gram FW. The MOPS supernatant was assayed for β-glucanase activity using hydroxyethylated cellulose as the substrate according to Truelsen and Wyndaele (1991). Proteins expelled from the cell wall with LiCl were assayed for β-galactosidase activity using p-nitrophenyl-galactoside as substrate and for endo-PG

activity using gel permeation chromatography with Superrose 6 (Pharmacia). Isolated total RNA was reverse-transcribed using random hexamer primers. Endo-PG's and β-glucanases were cloned using nested PCR and degenerated primers. The primer design was based on published amino acid sequences.

We found much β-glucanase activity in the DZ during the period of cell degradation (i.e. from five weeks after anthesis). Chromato-focusing identified a pI activity of 9.3 for the β-glucanase isoform in the DZ. There was less activity in the PW. Seven β-glucanase classes were identified using amplified DNA fragments and the low sequence homology between the classes indicates that each is encoded by a different gene. One β-glucanase class was found only in the DZ and may be a good candidate for isolation of a DZ-specific promoter and for the construction of an anti-sense gene.

Extracts from DZ and surrounding tissue, six and a half week after anthesis were found to contain a β-galactosidase with specificity for high molecular weight polymers extracted from rape cell walls. The extract was found to depolymerise citrus pectin completely and was active towards pure polygalacturonate. This demonstrated the presence of enzymes capable of degrading the middle lamella. We have identified six different classes of endo-PG, three of which were found to be localised in the pod wall and one only in the DZ.

A cDNA library has been constructed and screened for full length glucanase and endo-PG clones. This work complements the related programme which aims to identify the genes associated with pod development.

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