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GLUCOSINOLATES IN THE POLLEN OF RAPESEED AND INDIAN MUSTARD

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Key Word Index—*Brassica napus*; *B. juncea*; Cruciferae; rapeseed; Indian mustard; pollen; seed; glucosinolates.

Abstract—The glucosinolate composition of the pollen from rapeseed (*Brassica napus* cv Midas) and Indian mustard (*B. juncea*. cv Early Yellow) was investigated by HPLC. Rapeseed pollen was found to contain significant amounts of 2-hydroxybut-3-enyl-, 2-hydroxypent-4-enyl-, pent-4-enyl-, and 2-phenylethylglucosinolates. Trace amounts of but-3-enyl-, 3-indolylmethyl-, 4-methoxy-3-indolylmethyl-, and 1-methoxy-3-indolylmethylglucosinolates were also observed. Indian mustard pollen contained predominantly prop-2-enylglucosinolate, with minor amounts of 2-hydroxybut-3-enyl-, 2-hydroxypent-4-enyl-, but-3-enyl- and 2-phenylethylglucosinolates. Structural confirmation by GC/MS was obtained for three major rapeseed pollen glucosinolates and four major mustard pollen glucosinolates. Two unidentified glucosinolate-like compounds were also observed in rapeseed pollen. One of these compounds was also found in Indian mustard pollen, but was not observed in seed of either species. Comparison of seed and pollen glucosinolate composition is discussed in relation to the potential of pollen selection for low glucosinolate varieties.

INTRODUCTION

Recent studies of *Tradescantia* and *Zea* have indicated that at least 85% of the genes expressed in the diploid plant may also be expressed in its haploid pollen [1, 2]. If appropriate biochemical markers can be identified, it may be possible to use pollen selection as a new and efficient screening procedure in plant breeding programs [3]. Some interesting qualitative correlations exist between the fatty acid composition of pollen and seeds of rapeseed (*Brassica napus*), and these could form the basis of such a selection method for seed oil quality [4]. The present study has addressed glucosinolate content, an important chemical parameter of seed meal quality in cruciferous oilseed crops [5]. Glucosinolates are distributed throughout the vegetative and reproductive tissues of rapeseed and Indian mustard [6, 7]; however their presence in pollen of any species has not been reported. Seed and pollen samples of rapeseed and Indian mustard (*B. juncea*) were analysed using HPLC and GC/MS, to determine whether any qualitative chemical correlations exist that could form the basis for the development of quantitative probes for the identification of low glucosinolate cultivars through pollen selection.

RESULTS

Seed and pollen from rapeseed and Indian mustard were analysed by HPLC for their glucosinolate content. The identification of glucosinolates (Table 1) was based on HPLC retention times of authenticated desulphoglucosinolate standards. Identification of three of the major glucosinolates in rapeseed pollen and four of the major glucosinolates in Indian mustard pollen was also confirmed by GC/MS with reference to both electron impact and chemical ionization spectra.

The seed glucosinolate composition of rapeseed was similar to that previously reported by Sang *et al.* [8], but with the additional observation of trace amounts of 4-methoxy-3-indolylmethyl- and 1-methoxy-3-indolylmethyl-glucosinolates. In comparison, the HPLC profile of rapeseed pollen contained major peaks corresponding to 2-hydroxybut-3-enyl-, 2-hydroxypent-4-enyl-, pent-4-enyl-, 3-indolylmethyl- and 2-phenylethylglucosinolates. Trace amounts of but-3-enyl- and 4-methoxy-3-indolylmethyl glucosinolates were also observed in the pollen profile (Table 1).

TMS derivatives corresponding to 2-hydroxybut-3-enyl-, pent-4-enyl-, and 2-phenylethylglucosinolates, three of the major glucosinolates detected in this pollen sample, were obtained and subsequently identified by GC/MS. Also observed in the rapeseed pollen profile was an unknown peak at HPLC retention time of 17.4 min. Analysis of the TMS derivative by GC gave a single peak with a retention time identical to authentic pent-4-enyl desulphoglucosinolate. Subsequent GC/MS analysis indicated a molecular ion of m/z 688, by ammonia chemical ionization which corresponded to authentic pent-4-enyl desulphoglucosinolate. However, since authentic pent-4-enyl desulphoglucosinolate had a HPLC retention time of 23.3 min, the unknown was most probably 5-methylsulphinylpentyl desulphoglucosinolate, which could have formed the pentenyl derivative as a result of thermal degradation during GC/MS analysis.

Indian mustard seed was found to contain significant amounts of prop-2-enylglucosinolate. In addition, 2-hydroxybut-3-enyl-, 4-hydroxy-3-indolylmethyl-, 3-indolylmethyl-, 2-phenylethyl- and trace amounts of 1-

Table 1. The normalized glucosinolate composition of seed and pollen (% of total) from rapeseed and Indian mustard as determined by HPLC

Compound No.	Retention time (min)	Glucosinolate	Rapeseed Seed*	Rapeseed Pollen*	Indian mustard Seed*	Indian mustard Pollen†
1	4.5	2-Hydroxybut-3-enyl	64.9	6.2‡	0.6	4.2‡
2	5.5	Prop-2-enyl	2.0	<0.5	90.0	32.2‡
3	11.0	2-Hydroxypent-4-enyl	3.7	7.6	nd	1.6
4	16.3	But-3-enyl	17.3	<0.5	1.7	4.1‡
5	17.4	Unknown	nd	3.7	nd	nd
6	18.3	4-Hydroxy-3-indolylmethyl	5.0	nd	4.1	nd
7	24.7	Pent-4-enyl	4.5	2.5‡	nd	nd
8	31.3	3-Indolylmethyl	0.6	<0.5	1.2	nd
9	34.9	2-Phenylethyl	1.7	3.7‡	0.6	5.9‡
10	35.9	4-Methoxy-3-indolylmethyl	0.2	0.6	nd	nd
11	39.4	1-Methoxy-3-indolylmethyl	<0.5	<0.5	<0.5	nd
12	52.4	Unknown	nd	74.0	nd	52.0

*Mean of duplicate analyses.
†Single analysis.
‡Structure confirmed by GC/MS.
nd, Not detected.

methoxy-3-indolylmethylglucosinolates were also observed. These glucosinolates have been reported previously in mustard seed [7], but differed in relative amounts for the cultivar studied.

Indian mustard pollen was found to contain several seed glucosinolates, namely a predominance of prop-2-enylglucosinolate, with lesser amounts of 2-hydroxybut-3-enyl-, but-3-enyl- and 2-phenylethylglucosinolates. In contrast Indian mustard pollen did not contain any 4-hydroxy-3-indolylmethylglucosinolate, and in addition, gave a peak corresponding to 2-hydroxypent-4-enylglucosinolate (Table 1).

Unique to the pollen HPLC profiles of rapeseed and Indian mustard was the presence of a significant unknown peak at an approximate retention time of 52.0 min. This peak contributed 74% of the apparent glucosinolate content of rapeseed pollen and 52% of the apparent glucosinolate content in Indian mustard pollen. Analysis of its TMS derivative by gas chromatography yielded a variable number of closely eluting peaks, suggesting thermal degradation. This has been reported as characteristic of glucosinolates containing a terminal methyl sulphinyl group [9]. The electron impact mass spectra of these derivatized peaks contained ions characteristic of silylated desulphoglucosinolates [10]; however ions from the aglucone moiety were not present. Further investigations are necessary before the structure of this compound can be confirmed.

DISCUSSION

The results of the present study confirm the presence of glucosinolates within the pollen grains of *Brassica* species. This is the first report of any such findings and is of significance in relation to the potential for pollen selection for low glucosinolate varieties. The total glucosinolate content of pollen was less than 10% (fr. wt) of the level found in corresponding seeds. Qualitative comparisons indicate that both rapeseed and Indian mustard pollen contained several major seed glucosinolates that could be targeted in pollen selection. In rapeseed these were 2-hydroxybut-3-enyl-,2-hydroxypent-4-enyl-, 2-phenylethyl- and pent-4-enyl-glucosinolates. In Indian mustard pollen, prop-2-enyl-, but-3-enyl- and 2-phenylethylglucosinolates are present.

Both rapeseed and Indian mustard pollens appear to contain predominantly glucosinolates with an aliphatic side chain. The indole glucosinolates, namely 4-hydroxy-3-indolylmethyl- and 3-indolylmethyl-glucosinolates were either absent or very minor constituents. This contrasts with vegetative tissues where 3-indolylmethylglucosinolate is present in significant amounts at flowering, and with seed tissue where significant amounts of 4-hydroxy-3-indolylmethylglucosinolate can be found [11]. This appears to be indicative of a difference in either biosynthesis or metabolism of these compounds in pollen grains compared to other tissues, and may be a further example of the operation of pollen specific genes, such as those observed in *Tradescantia* and *Zea* [3].

In view of the relatively low concentration of glucosinolates within pollen grains and the difficulties in obtaining large amount of pollen, structural confirmation of pollen glucosinolates was a significant achievement. Further quantitative analysis is now necessary to establish whether relative amounts of glucosinolates in pollen can be correlated with the relative levels of glucosinolates in seed and thereby provide a basis for pollen selection. It will also be necessary to determine where the glucosinolates are located within the pollen grain and whether they are accessible, for example, to immunoassays or histochemical stains.

EXPERIMENTAL

Plant material. Field plots of rapeseed (*Brassica napus* L. cv. Midas) and Indian mustard (*B. juncea* Coss. cv. Early Yellow), were grown at the Victorian Crops Research Institute, Horsham, Australia. Pollen and seed samples were collected from the same plots in September and December 1985 respectively. Pollen samples were stored in liquid N_2 until use.

Glucosinolate extraction. Seed samples (200 mg) were extracted and analysed as ref. [12]. Pollen samples (125–300 mg fresh wt) were extracted overnight in 3.0 ml of $CHCl_3$–MeOH (2:1) as outlined in ref. [4]. They were then partitioned with 2.0 ml of hexane–H_2O (1:1) and centrifuged to separate the components into two phases. the upper MeOH–H_2O phase was transferred to a 10 ml vial and the MeOH evaporated under a stream of N_2 at room temp. The remaining aq. portion was then transferred to a graduated centrifuge tube and the protein material pptd by adding 0.25 ml of a 0.5 M solution of Pb and Ba acetate in a final volume of 5.0 ml.

Glucosinolate desulphation. Following centrifugation, 4.0 ml aliquots of the supernatant were diluted to 20.0 ml with H_2O, then added to 100 mg columns of DEAE-Sephadex A-25, prepared as described previously [12]. The eluant from these desulphating columns was freeze-dried using a Dynavac FD5 freeze-drier coupled to a D series 250/150 cc pump. The lyophilysed extract was then reconstituted in 0.5 ml of H_2O and analysed by HPLC according to Sang and Truscott [12]. The detector sensitivity was set at 0.05 AUFS for the analysis of pollen. Desulphoglucosinolate standards were prepared and authenticated as described previously [7, 11, 12].

Separated HPLC fractions were freeze-dried, transferred to 1.0 ml Reacti-vials, then silylated to their trimethylsilyl (TMS) derivatives. Silylation (120°, 30 min) was carried out using 30 μl Pyridine-BSFTA-TRISIL (25:10:1). Samples were initially analysed by GC, then by GC/MS. GC analysis of the TMS derivatives of desulphoglucosinolates was performed on a Packard 419 gas chromatograph, fitted with a BP5 capillary column. The helium flow rate was 4.0 ml/min. The temperature was programmed to increase from 200–300° at 5°/min. GC/MS identification was performed as outlined by Minchinton *et al.* [11].

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