

## CHARACTERIZATION OF ALLERGENS FROM RAPESEED POLLEN AND SEED (Brassica napus)



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**II – IMMUNE DETECTION OF ALLERGENS AFTER IEF III – STUDY OF ALLERGENS BY 2D-GEL ELECTROPHORESIS** Siver staining Immunoblo The first 31 patients sera containing IgE were classified by decreasing ELISA values order, and tested by immunoprint, for their recognition of water-soluble or TUC 4.75 5.66 5.92 6.45 7.30 8.30 4.75 5.65 5.65 5.65 5.65 5.65 7.30 8.30 8.30 8.30 8.30 Brassica napus pollen or seed extract. Among them, 13 (arrows) were positive, and Water-soluble ii. 111 pollen extract kDa TUC pollen extract 1 - Profilin 91 -Detection o 2 - Calcium binding protein 3 - Cobalamin independent methionine synthetase allergen by serum Nº 28 3 4 - Pectinase (polygalacturonase 5 - I TP1 (I inid Transfert Protein) 6 - Receptor-like protein kinase 7 - Berberin bridge enzyme Chardin et al. Allergy 2003; 58:407-41 Chardin et al. Int. Arch. Allergy & Immunol 2001; 125:128-134 5.92 475 565 565 565 565 565 550 5645 830 830 830 TUC LD. pollen extract 94 -Detection of allergens 67 by serum N° 28 20.1-- 475 - 475 - 485 - 485 - 485 - 485 - 565 - 565 - 830 - 830 5.92 5.92 3 3 THC seed extract Water-soluble seed extract Detection of allergens by serum N\* 28 20.1-144-BY MASS SPECTROMETRY ...... TUC seed extract Detection of allergens by serum 67 . 30 -20.1 20.1-

> V - CONCLUSIONS: The major allergens from Brassica napus pollen and seeds, water-soluble or water-insoluble, are all different, based on their isoelectric points, molecular mass and function. We have identified 9 new allergens or isoallergens among the water-insoluble proteins from Brassica napus seeds, already known by their sequence in protein data bases but so far not referred as allergens.

INTRODUCTION: Rapeseed (Brassica napus), is the main oleaginous seed cultivated in Europe and used to produce seed rape oil for human consumption. Cattle-cake is used for animal consumption and some Brassica napus seed components are found in beauty care products, washing powder and also in offset ink, fuel, lubricant,

Four hundred out of 2000 sera of allergic patients were tested by ELISA. Fifty out of 400 sera had IgE antibodies anti water soluble proteins of rapeseed. Among these 50 sera, 31 were chosen for their high amounts of IgE, and were used to study water and waterinsoluble proteins from pollen and seed extracts separated by IEF. In order to sharpen these identifications. we used 2D electrophoresis, immunoblots and mass spectrometry.

#### I - MATERIAL & METHODS

Brassica napus SEED AND POLLEN EXTRACT IN : - H<sub>2</sub>O

- TUC (2M Thiourea + 7M Urea + 5% CHAPS)

PROTEINS

Quantitation : Bradford (Coomassie) Characterization : IEF (pH 2-11) 2D del Electrophoresis

#### ALLERGENS

IgE immune detection :

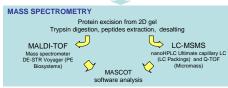
1.- After blotting, (blot) incubating with allergic patient sera 2.- Alkaline phosphatase labelled goat antibody anti-human IgE

Alkaline phosphatase detection

HUMAN SERA TESTED

From allergic patients sensitized to grass and birch pollen (specific IgE) Characteriza

Screening by ELISA of water soluble Brassica napus seed and pollen extract



# IV – IDENTIFICATION OF WATER-INSOLUBLE SEED ALLERGENS

showed a great heterogeneity

Water soluble pollen extract

Water soluble seed extract

pl

= 4.75

5.65

= 5.92 6.45

- 8.3

=<sup>4.75</sup><sub>4.85</sub>

- 5.65 - 5.92 - 6.45

Spot	app Mw (kD)	app pl	Identified proteins	Theoritical Mw (D)	Theoritical pl
1	80-81	9.4- 9.5	myrosinase [Brassica napus]	62746	8.70
2a	43	8.4	myrosinase-associated protein	41810	9
2b	43	9	myrosinase-associated protein	41797	8.47
2c	43	9.4- 9.5	myrosinase-associated protein	41797	8.47
3a	27.9	6.2	cruciferin cru4 subunit [Brassica napus]	45512	8.84
3b	27.9	6.9	cruciferin cru4 subunit [Brassica napus]	45512	8.84
4a	23	11.7- 12	napin large chain L2B ou L2A=calmodulin antagonist/calcium-dependent protein kinase substrate	10229 + 20851 + 9809	9.32 + 7.56 + 9.13
4b	19.3	11.7- 12	Napin + allergen sin a 1.0105 [Sinapis alba]	20851 + 16407	7.56 + 8.54
5	19.3	5.8	seed storage protein beta-chain 6 - Arabidopsis thaliana (fragment) + cruciferin cru2/3 subunit [Brassica napus]	1086 + 12000	4.53 + 9.30

Excised proteins from the silver-stained 2D gel (circles on TUC seed extract figure) were analysed by LC-MS. Identified proteins were listed in the table and localized on the corresponding blot (circles)