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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 water-insoluble 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 :**

- H₂O
- TUC (2M Thiourea + 7M Urea + 5% CHAPS)

PROTEINS

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

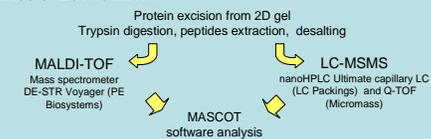
ALLERGENS

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

HUMAN SERA TESTED

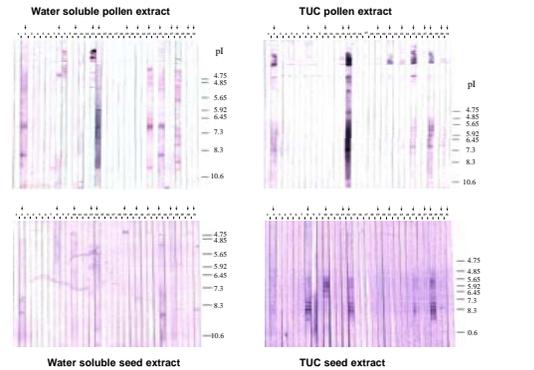
- From allergic patients sensitized to grass and birch pollen (specific IgE)
- Characterization:
- Screening by ELISA of water soluble *Brassica napus* seed and pollen extract

MASS SPECTROMETRY



II - IMMUNE DETECTION OF ALLERGENS AFTER IEF

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 *Brassica napus* pollen or seed extract. Among them, 13 (arrows) were positive, and showed a great heterogeneity

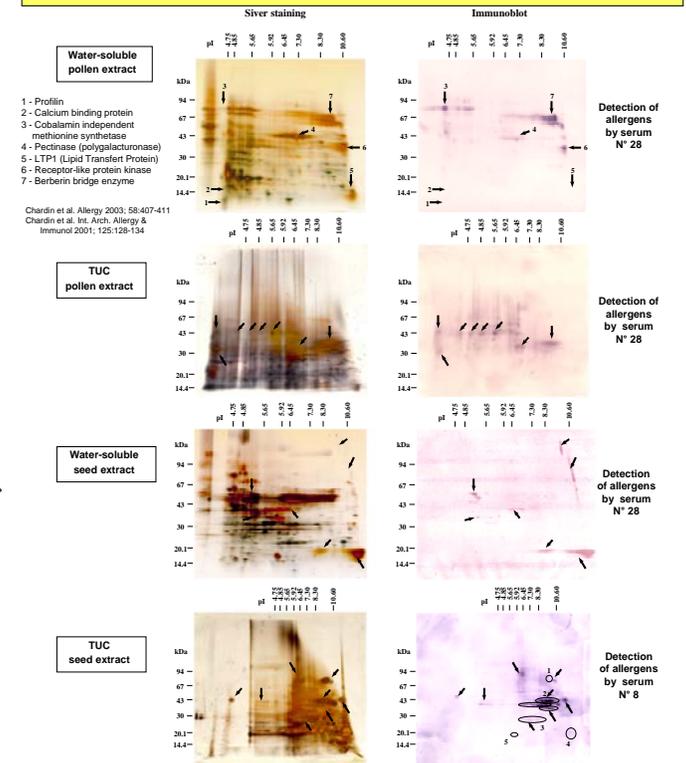


IV - IDENTIFICATION OF WATER-INSOLUBLE SEED ALLERGENS BY MASS SPECTROMETRY

Spot	app Mw (kD)	app pI	Identified proteins	Theoretical Mw (D)	Theoretical pI
1	80-81	9.4-9.5	myrosinase [<i>Brassica napus</i>]	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 [<i>Brassica napus</i>]	45512	8.84
3b	27.9	6.9	cruciferin cru4 subunit [<i>Brassica napus</i>]	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 [<i>Sinapis alba</i>]	20851 + 16407	7.56 + 8.54
5	19.3	5.8	seed storage protein beta-chain 6 - <i>Arabidopsis thaliana</i> (fragment) + cruciferin cru2/3 subunit [<i>Brassica napus</i>]	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)

III - STUDY OF ALLERGENS BY 2D-GEL ELECTROPHORESIS



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.