Genetic variability of rapeseed protein composition.

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ABSTRACT

The genetic variability of the seed protein composition was studied in 64 cultivars and lines of rapeseed (*Brassica napus L.*) grown under field conditions. These varieties belonged to three main classes: rich in both erucic acid and glucosinolates (++) , low in erucic acid and rich in glucosinolates (0+) and low in both erucic acid and glucosinolates (00). The methodology developed was based on standardised extraction and chromatographic procedures coupled to statistical analysis. A rather high variation of the cruciferin and napin contents was found, respectively in the ranges of 32-53% and 25-45% of the seed proteins respectively. The cruciferin/napin ratio was significantly higher for the 00 varieties compared to ++ ones. On this basis, the three genetic families could be well discriminated by statistical analysis. The genetic linkage between these different rapeseed varieties was also complementary estimated through DNA fingerprinting based on microsatellite analysis. A total of 34 varieties were fingerprinted using 8 microsatellite primer sets. A comparison of the data from DNA fingerprinting and protein profiling of these varieties has been carried out to establish the most reliable estimate of genetic variability between the different cultivars.

On the basis of these data, and of the differences between properties of cruciferins and napins, enhancement in the protein quality of seeds by breeding could be suggested for producing added-value meals.

Key words: rapeseed, protein, DNA fingerprinting, cruciferin , napin, genetic variability.

INTRODUCTION

The breeding objectives for the seed quality of rapeseed (*Brassica napus L*.) were mainly concerned during the last twenty years with the quantitative and qualitative improvement of the oil fraction and with the decrease of glucosinolates in the meal . The low value of the meal, which is the by-product of the oil extraction but consists 40 to 45% of proteins, is indicative of the limited interest for the seed protein fraction. A higher profitability of the rapeseed integrated chain would only be achieved by developing new higher-value applications for these oilmeal protein fractions. Some recent results highlight the feasibilty of protein extraction processes, and the economic potential of the resulting innovative protein products, especially in the non-feed area (Sanchez-Vioque et al., 2001, Gerbanowski et al. 2003).

Besides, the prospective assays on alternative aqueous oil extraction processes have shown that some bottlenecks are due to the difficulties in separating the oil fraction from the proteins, due to their ability in stabilizing emulsions. Consequently, the development of innovative technology and products from rapeseed needs to take into account more accurately the protein fraction of the seeds. As a basis for such prospective developments, our study was aimed at establishing for the first time the genetic variability of the protein composition from *Brassica napus L.*.

MATERIALS AND METHODS

Materials: The seed protein composition was studied on 64 rapeseed genotypes (*Brassica napus L.*) grown in field conditions by INRA Rennes. These varieties belonged to three main classes: those rich in both erucic acid and glucosinolates (++) , low in erucic acid and rich in glucosinolates (0+) and low in both erucic acid and glucosinolates (00).

Protein analysis : The seeds were ground, using a freeze mill (6700 Spex) in the presence of liquid nitrogen. The resulting flour (1g) was defatted by dichloromethane (10mL) and then extracted by Tris-HCl buffer 50mM, pH 8.5, NaCl 0.75M (ratio 1g flour for 10mL buffer). The extracted pigments were eliminated from the crude extract by desalting chromatography. The uncoloured samples loaded on a Superdex 75 are fractionated into three main peaks corresponding to cruciferin, napin and lipid transfer protein. These fractions were quantified from the peak areas, corrected from the respective extinction coefficient at 280nm determined for the three proteins. Each cultivar was analysed in triplicate from three different protein extracts. Statistical analysis of the chromatograms was performed by principal component analysis (PCA) and the corresponding distribution map were established for the 64 samples.

DNA fingerprinting*:* The genetic variability within a cultivar, and between different cultivars , was determined by DNA fingerprinting, based on microsatellite allele sizing. DNA isolated from either individual seeds of the variety 'Express', or de-oiled rapeseed meals from the 34 different cultivars and lines was used for microsatellite analysis. Each meal comprised of seeds collected from a number of plants of a given variety. The primers used for microsatellite analysis (Table1) were derived from several members of *Brassiceae* family including *Brassica napus L.* (Uzunova and Ecke, 1999, Mitchell et al., 1997). These primers were optimised for routine use.

Table 1: Sequence of primers used for microsatellite analysis (H,T and F represent the ABI dyes Hex, Tet and Fam respectively)

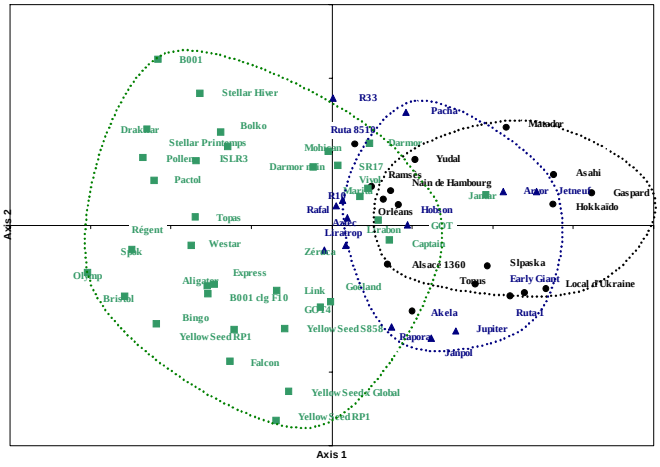
Primer	Sense primer	Antisense primer
BN01	H-AAAAATAGAAAGTTTGGAAGG	CCAACACACCTTTTACTCTT
BN02	T- TCGACATGGATTCTACCAAA	GAACTTGCAAGCTGCAATTA
BN04	H-GATTGAACACCCCTAGTGAG	TATCAAAAACCCCAAGATTG
BN07	H-AAACCTCCTCAAAAACCCCTAAACG	TCCCCTCTTTCCTCTCTCTCTAGGC
BN08	T-GCCGTTCTAGGGTTTGTGGGA	GAGGAAGTGAGAGAGGGGGAAATCA
BN35D	F-GCAGAAGGAGGAGAAGAGTTGG	TTGAGCCGTAAAGTTGCTCACCT
BN59AI	T-TGGCTCGAATCAACGGAC	TTGCACCAACAAGTCACTAAAGTT
BN83/1	F-GCCTTTCTTCACAACTGATAGCTAA	TCAGGTGCCTCGTTGAGTTC

PCR amplification of microsatellite alleles was carried out either using DNA extracted from individual seeds or 100 mg of deoiled meal using 'NucleoSpin Plant Kit' (Macherey-Nagel). The PCR conditions were 94°C for 2 minutes, followed by 40 cycles at 94°C for 45 seconds, annealing at corresponding temperatures for 30 seconds, and extension at 72°C for 1 minute. This was followed by final extension at 72°C for 5 minutes and storage at 4°C. The PCR products obtained by microsatellite method were separated by electrophoresis and size of each product determined on an automated DNA analyser (ABI-Prism-377).

RESULTS

Protein analysis: For the 64 genotypes, the protein content of the seeds comprised in the range 18.8-25.2% d.m., the new varieties being generally characterized by a lower protein content. The cruciferin and napin contents were comprised between 32-53 % and 25-45% of the seed proteins respectively. For most of the varieties the cruciferin was the major protein and for some the cruciferin/napin ratio reached 2.

On the PCA factorial map, established for all of the genotypes, the older varieties rich in both glucosinolates and erucic acid were very clearly discriminated from the recent double null ones.



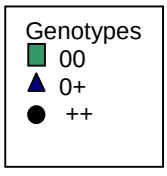


Figure 1: PCA factorial map of composition data and chromatogram discriminating the rapeseed genotypes.

The main discriminating factor, mainly represented by axis 1, was the cruciferin/napin ratio. It means that the older varieties (++) were richer in napin in opposite to the double null ones which contained cruciferin as a major family.
This result was confirmed on peculiar genetically derived genotypes. As an example, Darmor and Darmor nain which are double null varieties derived from Jetneuf (0+) and primarily from Gaspard (++) have a cruciferin/napin ratio at 1.3 and 1.1 compared to 0.8 and 0.7 respectively in the case of Jetneuf and Gaspard. The proximity of genetically close varieties or lines on the map, like Darmor and Darmor nain, Samouraï and ISLR3, or the yellow seeds varieties suggested that the protein composition of the seeds is mainly ruled genetically and rather less by environment factors. That seems to be confirmed by the proximity of the two Stellar samples, respectively grown in spring and winter. To establish the genetic proximity of these samples, a microsatellite analysis was performed on 34 varieties and a dendogram was built.

Microsatellite analysis: The data obtained by microsatellite analysis showed that there was no intra-varietal variability within the variety 'Express', whilst there was variability between 34 other rapeseed varieties. A matrix table based on allele sharing between the varieties was produced and used to build a dendogram (Fig. 2). It will be seen that the method successfully grouped together a number of genetically-close varieties, such as Darmor, Stellar and Yellowseed varieties. The genetic similarity between varieties obtained by microsatellite analysis also compared very well with similar data obtained by protein profiling of the varieties. Out of a total of 34 varieties tested, 22 grouped in a similar manner by both protein profiling and DNA analysis, whilst 12 varieties (Arvor, Link, Zeruca, Bristol, Pacha, B001, ISLR3, Pollen, Matador, Gaspard, R33 and Hokkaido) were grouped differently by the two methods.

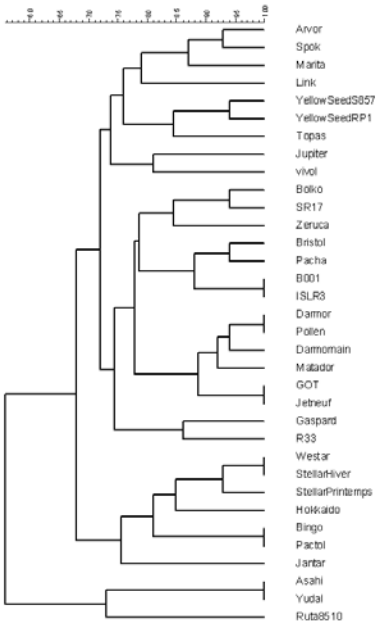


Figure 2: Dendogram of rapeseed genotypes

DISCUSSION AND CONCLUSION

From these results, it can be clearly seen that the breeding programmes devoted over the past twenty years to improve the oil fraction in the oil seed rape not only induced a decrease in the seed protein content, but also a concomitant enrichment in the level of cruciferins.The similarities found by genetic analysis and the protein data, further suggest that the genetic factors mainly govern the protein composition. It also emphasises that breeding efforts could be devoted to the improvement of the protein quality in seeds for adapting rapeseed meal to derive protein enriched products for specific higher added-value uses.

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