NAPUS 2000 – a research programme for the improvement of the whole rapeseed

Gunhild Leckband[1], Hilke Rades[1], Martin Frauen[1], Wolfgang Friedt[2]
[1] Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, 24363 Holtsee, Germany, mail: NAPUS@npz.de
[2] Institute for Crop Science and Plant Breeding, Justus-Liebig-University, Heinrich-Buff-Ring 26-32, 25392 Giessen, Germany,mail: wolfgang.friedt@agrar.uni-giessen.de

ABSTRACT

NAPUS 2000 is a major German research project, sponsored since 1999 by the German Federal Ministry of Research (BMBF). This project comprises 19 partners (universities, food industry, plant breeders) which focus on the production of oilseed rape (*Brassica napus* L.) with improved nutritional value and on the use of the entire kernels for human nutrition. The content and / or quality of tocopherol, protein and lecithin are improved and long chain polyunsaturated fatty acids (LCPUFAs) and resveratrol are newly introduced. Traditional breeding methods as well as genetic engineering techniques are applied. In addition, processing trials, nutritional research, economic studies and socio-economical assessments are conducted.

Early results indicate that it is possible to integrate a number of new improved seed traits in traditional and / or transgenic oilseed rape. Traditional breeding programmes have already yielded lines with improved high-tocopherol content or improved protein composition; high-lecithin and specific-protein varieties are also in development. Genetically modified varieties enriched in long-chain polyunsaturated fatty acids, tocopherols, resveratrol, lecithin or protein are in early stages of development.

The application of additional compounds of the seeds for human nutrition would increase the added value of oilseed rape. So far the meal is used as high value compound in animal feeding even though there are some compounds in the meal which are suitable for food application, e.g. functional protein fractions.

Key words: Brassica napus – plant breeding –genetic engineering –food application – protein quality

INTRODUCTION

Oilseed rape is one of the most important oil crops in Europe, with a planted area of about 3.07 mio ha in 2002 in EU 15 (62% of the total oil crop area of 4.9 mio ha). Rapeseed oil derived from double-low oilseed rape (*Brassica napus* L.) varieties ('canola') is classified as one of the healthiest vegetalbe oils for human consumption (Trautwein, Erbersdobler; 1998). Furthermore the meal is a high-value by-product used in animal feed due to its high energy content and the high amino acid score of the protein.

Future markets will increasingly demand food with a positive influence on human health. For this reason, the co-operative project called "NAPUS 2000 - Functional Food from transgenic rapeseed " was formed in 1999 by a consortium of 19 partners. These partners include breeding companies, universities, research institutions and processing companies (small/medium sized enterprises and international companies). Norddeutsche Pflanzenzucht Hans-Georg Lembke KG is responsible for the management of the project.

The project's aims are two fold: to improve the nutritional value of oilseed rape, and to use whole rapeseed kernels for human food applications. The research targets can be divided into naturally-occurring seed components, such as tocopherols, proteins and lecithin, and newly introduced components, such as the antioxidant resveratrol and long-chain polyunsaturated acids (LCPUFA). Traditional breeding methods as well as genetic engineering techniques are included in the project. Furthermore, processing techniques are being optimised to handle the new seed types and to isolate new fractions such as LCPUFA and native protein.

The partnership

The 19 partners of NAPUS 2000 conduct research in several fields in parallel. This structure allows for an early exchange of results between the partners and thus leads to efficient work plans. As an example, processing trials can identify substances that might decrease the quality of a newly-introduced ingredient (for instance, the phenolic acid sinapine reduces the quality of protein isolates derived from rapeseed). Thus the scientists can concentrate on eliminating this substance by genetic engineering while the breeders create new breeding programmes aimed at this target. The fields of research conducted in parallel are:

1. discovery, characterisation, isolation and transfer of candidate genes
2. development of well-performing varieties with important agronomic traits by traditional breeding and genetic engineering
3. processing trials on new seed types or model systems
4. nutritional studies of the new types
5. economic studies on the potential of new types of rapeseed

The first step in the project is to obtain scientific proof of concept without being limited by constraints such as those imposed by patent applications. To get an idea of the technical and nutritional properties of the transgenic rapeseed, analyses with model substances are undertaken at the same time.

After proving the feasibility for production of a novel trait such as a reduced sinapine content, further investigations are started with the objective of patent applications and licensing. Gene constructs consisting of specific seed promoters and optimised genes of interest are transferred into oilseed rape, preferably by protoplast transformation. If these investigations reveal 'lead event' rapeseed lines, these are integrated into well-adapted plant material which was specially pre-selected for the focussed target quality. In field trials the material is multiplied and selected for commercial agricultural applications.

The produced seed will be used for extensive processing trials to provide knowledge about the localisation (extraction phases) and extractability of the target substances in the seeds. Nutritional and socio-economic studies will accompany research during the whole project, using model substances and the seed harvested.

Protein

The use of compounds of the meal, e.g. protein or lecithin, not only for animal nutrition but also for food application would increase the added value of oilseed rape for plant breeding companies, farmers and industry. The protein fraction of rapeseed is known for its high nutritional value, but it has not yet been used for human nutrition. So far most of the vegetable protein used for food application is derived from soya. First results of the project have shown that protein isolated from rapeseed could be suitable for food application, but there are some negative characteristics which would limit its use (Natsch, Wäsche, 2003). The protein isolates are more or less coloured and can have an undesireable smell, caused by different compound e.g. glucosinolates. Moreover seed components such as sinapine and tannins are complicating the extraction of functional proteins, therefore low-sinapine and yellow-seeded rapeseed genotypes will be developed.

The sinapine metabolism is an important field of research to increase the quality of rapeseed protein (Hüsken et al. 2003). Within the NAPUS project a major gene of sinapine synthesis was identified, other genes are still to be found (Milkowski et al. 2000). The aim is to repress these genes by antisense or co-suppression strategies within the project.

Studies are carried out to gain knowledge about inheritance and to facilitate the breeding process, natural variation has already been detected. Parallely extensive processing trials are carried out to identify which parameters optimise protein quality (e.g. function, smell, colour). Five different processes of oil and protein extraction are on focus in the ongoing work to develop a new protein product based on oilseed rape.

Yellow seed coat – a favourable novel quality

The black seed coat colour of canola results from a number of different polyphenolic components which hinder the extraction of pure seed components like proteins and lecithin. The partners in NAPUS 2000 developed two main strategies to reduce these polyphenolic

components: one strategy is the breeding of yellow seeded oilseed rape, the other main strategy is to reduce specific substances with a negative influence on extractability by genetic engineering. Also a technical approach is investigated within NAPUS 2000. Dehulling rapeseed before crushing could be an appropriate way to increase protein quality derived from black seeded rapeseed. Nevertheless, dehulling rapeseed to a satisfactory extent and chrushing dehulled rapeseed requires specific techniques. This is the aim of several processing trials.

Breeding yellow seeded lines is difficult because the trait 'seed coat colour' is influenced by several genes. Furthermore environment has a strong influence on the expression of this trait. Besides this the breeder must take into account that the polyphenolic components in the seed coat have an important function as a defence against disadvantageous environmental influences during storage and during seed germination.

Nevertheless, a lot of progress has been achieved in this area and conventional breeding material has been propagated for processing trials in NAPUS 2000 with respect to extractability and quality of the protein and lecithin.

Perspectives

After about 3.5 years of work in NAPUS 2000 the first results indicate that it is possible to integrate a number of new and improved seed traits in traditional and / or transgenic oilseed rape.

Traditional breeding programmes concerning tocopherols, sinapine and protein have been started successfully and lines with improved tocopherol content or yellow seeded lines might be ready for official testing in a few years. In the case of lecithin the breeding companies need more time to evaluate different lines and to create appropriate breeding schemes.

The project parts using genetic engineering need more time to reach the targets. In the case of LCPUFAs, tocopherol and protein, this is due to the complex metabolism. For lecithin and resveratrol some transgenic plants have already been generated, analysed and selected for further breeding steps. Parallel screenings of conventional breeding material revealed oilseed rape lines that are well-suited for further approaches to integrate optimal transformants in adapted plant material. Transgenic lead events will be selected for the initiation of breeding, or at least optimised constructs are ready for transformation into rapeseed.

Acknowledgements

The authors acknowledge the Federal Ministry of Research and Education of Germany (BMBF) for supporting the project as well as all partners of NAPUS 2000. These are: Norddeutsche Pflanzenzucht Hans Georg Lembke KG, Deutsche Saatveredelung Lippstadt-Bremen GmbH, Saaten-Union Resistenzlabor GmbH, Raiffeisen Ölsaatenverarbeitungs GmbH, BASF AG, Numico Research Group Germany, Unilever Bestfoods Deutschland GmbH, Degussa Texturant Systems GmbH & Co. KG, Rovita GmbH für Milch- und Stärkederivate, University of Hamburg, University of Aachen, Institute of Plant Biochemistry Halle, Justus Liebig University of Giessen, Georg August University of Göttingen, Christian-Albrechts-University of Kiel, Fachhochschule Münster, Fraunhofer Institute for Toxicology and Experimental Medicine, Fraunhofer Institute for Process Engineering and Packaging.

REFERENCES

Hüsken, A., Milkowski C., Becker H., Möllers C. (2003): Metabolic engineering of the sinapic ester content in oilseed rape (Brassica napus). GCIRC Congress 2003 Kopenhagen.

Milkowski, C., Baumert, A., Strack, D. (2000): Cloning and expression of a rape cDNA encoding UDP-glucose:sinapate glucosyltransferase (SGT). Planta 211: 883-886

Natsch, A., Wäsche, A. (2003): Rape Protein Products as Food Ingredients. GCIRC Congress 2003 Kopenhagen

Trautwein, E.A., H.F. Erbersdobler (1998) Rapsöl – ein wertvolles Speiseöl. UFOP-Schriften, series No 6, p. 38-67. Union zur Förderung von Oel- und Proteinpflanzen e.V., Bonn.