**NAPUS 2000 Rapeseed (Brassica napus) breeding for improved human nutrition**

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**Auteur(s) :** Wolfgang FRIEDT, Martin FRAUEN, Gunhild LECKBAND, Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, D-24363 Hohenlieth, Germany.

**Summary :** Following a competition announcement of the Federal Ministry of Research and Education (BMBF) a project dealing with the improvement of the nutritional value of oilseed rape (Brassica napus) for food applications and human nutrition was worked out and started in autumn 1999. A number of partners (Figure 2) are carrying out a complex project reaching from the discovery, characterisation, isolation and transfer of genes of interest up to breeding of well performing varieties combined with important agronomic traits. Economic studies and processing trials as well as nutritional investigations of the new qualities are undertaken. B. napus seed quality aspects with respect to seed coat colour, oil composition, lecithin and protein fractions and antioxidants like tocopherols and resveratrol will be improved.

**Keywords :** Brassica napus, genetic engineering, tocopherols, lecithin, LCPUFA, resveratrol, sinapine, yellow seediness.

**Résumé :** A la suite d'un appel d'offres du ministère fédéral de la Recherche et de l'Éducation (BMBF), un projet d'amélioration de la valeur nutritionnelle du colza (Brassica napus) pour des débouchés alimentaires ou de nutrition humaine a été initié en automne 1999. Un projet intégré allant de la recherche, la caractérisation, l'isolement et le transfert de gènes d'intérêt en sélection, dans des variétés à haut potentiel et ayant des caractéristiques agronomiques intéressantes, est actuellement conduit par un groupe de partenaires (figure 2). Des études économiques, des essais, ainsi que des analyses nutritionnelles pour des qualités nouvelles sont conduites dans ce programme. Les aspects de qualité des semences en relation avec la couleur des téguments de la graine, la composition en huile, en lécithine ainsi que les fractions protéiques et les antioxydants, tels que les tocophérols et le resveratol, seront améliorés ches Brassica napus.

**Mots-clés :** Brassica napus, transformation génétique, lécithines, tocophérols, LCPUFA, resveratol, sinapine, graines jaunes.

ARTICLE

## Introduction - the vision

Rapeseed oil derived from current cultivars with 00-quality ("canola") is already classified as one of the healthiest vegetable oils [1]. Future markets will increasingly demand food with a positive influence on human health. By combining modern biotechnology like genetic engineering with classical plant breeding new innovations will produce this functional food.

The BMBF project NAPUS 2000 aims at a comprehensive utilisation of oilseed rape kernels as a whole in human health and nutrition. One main aspect in developing the concept and structure of NAPUS 2000 was to guarantee a comprehensive approach involving the whole process from gene isolation to a new product as mentioned before. The realisation of this concept is visualised in *Figure 1.*

Targets of the our investigations are different seed components: firstly native compounds like tocopherols, proteins and lecithins and, secondly, newly introduced substances like resveratrol and long-chain polyunsaturated fatty acids (LCPUFAs). To handle the new qualities and to carefully isolate fractions like LCPUFAs and native protein, processing techniques are correspondingly adapted. Furthermore, all improved or novel seed components are investigated in detail by food scientists and the food industry in order to produce healthy and save food from this new rapeseed qualities.

## The partnership

For the realisation of the mentioned complex tasks a consortium was arranged consisting of 17 quite different partners *(Figure 2)*. Scientists from universities and research institutes are involved in gene identification, isolation, transformation work, screening suitable genotypes and establishing highly efficient transformation protocols. They also undertake a more practical approach studying the inheritance of different seed traits, developing analytical calibrations for fast and precise screenings of newly generated seed material and investigating nutritional aspects. Also they are carrying out extensive small and large scale processing investigations aiming at the development of methods to isolate the different target substances of the rapeseed kernels carefully.

The commercial partners are working on applied approaches of seed production and processing. They are responsible for following steps in the production pipeline: (1) the selection of rapeseed genotypes suitable for the realisation of the novel seed components; (2) the integration and multiplication of transgenic and non-transgenic lines; (3) continued breeding and multiplication of the rapeseed materials; and (4) screenings of organisms used as potential gene sources. Furthermore, commercial partners are analysing the amount and quality of the target substances in the resulting fractions after crushing. This aims at additional nutritional and processing investigations with respect to the next steps towards the development of new marketable products.

## Aims and first results

### *Introduction of LCPUFAs into oilseed rape*

A novel plant oil quality which has been discussed intensively in the last years consists of a fatty acid pattern containing a significant amount of LCPUFAs which naturally occur e.g. in fish oils, are essential in human nutrition and are lowering the risk of coronary heart diseases [2, 3]. One aim of NAPUS 2000 is

the development of a rapeseed oil with a modified fatty acid pattern in order to increase the daily intake of the omega-3 fatty acids EPA (C20:5) and DHA (C22:6). Another goal is the production of an oil highly enriched in LCPUFA content to generate concentrates after further processing procedures.

The modification of a number of genes is necessary to produce the mentioned fatty acids in an oil crop [4-6]. The investigations will further indicate if it is possible to introduce the new genes in one cassette, in different constructs or whether it is better just to combine different lines each carrying one essential gene by conventional cross-breeding.

Selections of most suitable oilseed rape genotypes with regard to the fatty acid pattern can facilitate the achievement of the focussed targets *(Figure 3)*.

***Yellow seediness - a favourable prerequisite for novel qualities***

The typical, thick-walled and black seed coat of oilseed rape has an important function as a defence against disadvantageous environmental influences during storage and development of the seedling. But besides these positive functions the substances hinder the extraction of pure seed components like proteins and lecithin [7, 8].

One way to lower these effects is the breeding of yellow seeded oilseed rape, which is characterised by a thinner seed coat with less polyphenolic components. Due to a relatively complex inheritance and a strong environmental influence on the trait "seed coat colour" generating a true breeding yellow seeded oilseed rape line is difficult to achieve [9, 10]. Nevertheless, ongoing work is undertaken to genetically engineer oilseed rape to change the mentioned fractions itself or reduce further substances with a negative influence on extractability. First steps and results are described below.

***Lecithin***

The lecithin fraction is the sum of different polar lipids which can be sub-divided in phospholipids and glycolipids. Both groups are targets of different approaches to increase the lecithin fraction of oilseed rape.

Parallel to the molecular approaches different oilseed rape cultivars of different origin and seed coat colour are screened to obtain information about the natural variability of the lecithin content and composition in oilseed rape.

***Protein (Sinapine)***

The protein of natural rapeseed is known to be of a high nutritional value. But it is not used in human nutrition due to antinutritional seed components like sinapine and tannins complicating the extraction of functional proteins [7]. Therefore, low-sinapine rapeseed genotypes will be developed. Natural variation for sinapine content has already been detected. In addition, molecular work is carried out in the project to identify major genes of the sinapine metabolism with the aim of repressing these genes by antisense or co-suppression strategies.

***Tocopherols***

Tocopherols (TOC), e.g. vitamin E, are natural compounds in the seed-oil of rape and other oil plants [11-13]. Again, conventional breeding material has already been screened and selected for the improvement of this trait. The two main TOC isomeres, alpha - and gamma-TOC, seem to have different physiological functions. In NAPUS 2000 both isomeres are considered for different purposes: whereas alpha-TOC is well known for its highest vitamin E activity and is thus important for human nutrition and relevant because of various health implications [14], gamma-TOC plays a key role as antioxidant in the seed [13] and might become necessary in case of the production of vegetable oils rich in LCPUFAs and an increased phospholipid fraction to prevent these from being oxidised rapidly.

Different genes involved in the biosynthesis of tocopherols are identified, isolated and transferred into oilseed rape to increase the level of the respective TOC-isomere(s).

Additionally, a large-scale crushing experiment was undertaken to record the tocopherol flow within the crushing process and to identify key steps in handling a high-TOC rapeseed genotype.

***Resveratrol***

A further antioxidant is resveratrol, a polyphenolic substance naturally found in the skin of grapes (*Vitis vinifera* L.) acting there as an antifungal factor. In nutrition of mammals a moderate consumption has a potentially positive influence on cancer and cardio-vascular diseases [15, 16]. Nevertheless, the positive influence of resveratrol on human health is not completely clear up to now. Consequently, very extensive nutritional trials involving cell culture and animal feeding investigations with the help of model substances are going to be started soon.

In previous studies the synthesis of resveratrol in transgenic oilseed rape and cereals was already proven in order to increase the level of disease resistance in these cultivated plants [17, 18]. This was relatively simply achieved due to the natural availability of resveratrol precursors in the plant and the need for solely one enzyme, a stilbene synthase [19].

CONCLUSION

**... and perspectives**

After one year of NAPUS 2000 the first results indicate that it is possible to integrate a number of new and improved seed traits in transgenic oilseed rape. In the fields of LCPUFAs, tocopherol and protein it will take some time to realise the focussed targets due to the more complex traits and difficult tasks. On the contrary, some first transgenic plants harbouring genes to modify lecithin and resveratrol metabolism have already been generated. Parallel screenings of conventional breeding material revealed oilseed rape lines being well suited for further approaches to integrate optimal transformants into adapted plant material.

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Illustrations

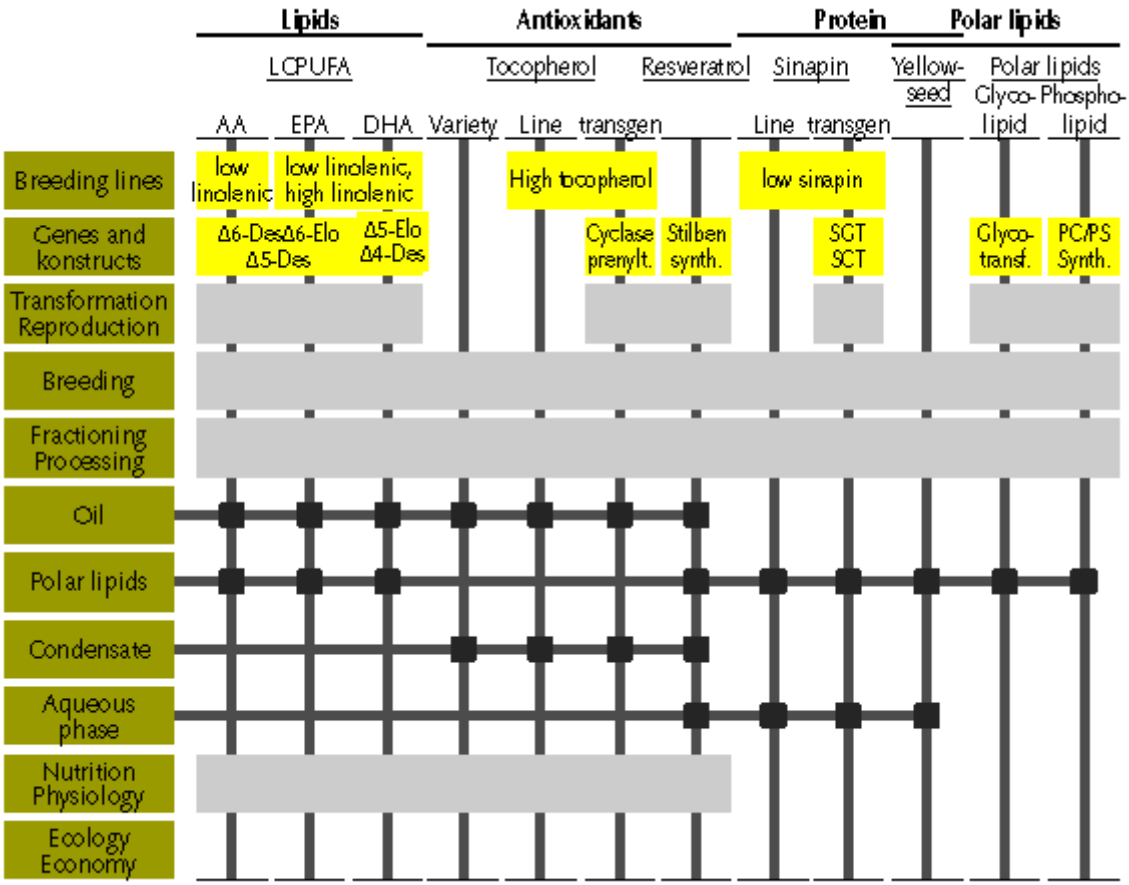


Figure 1. *Scientific concept in NAPUS 2000.*

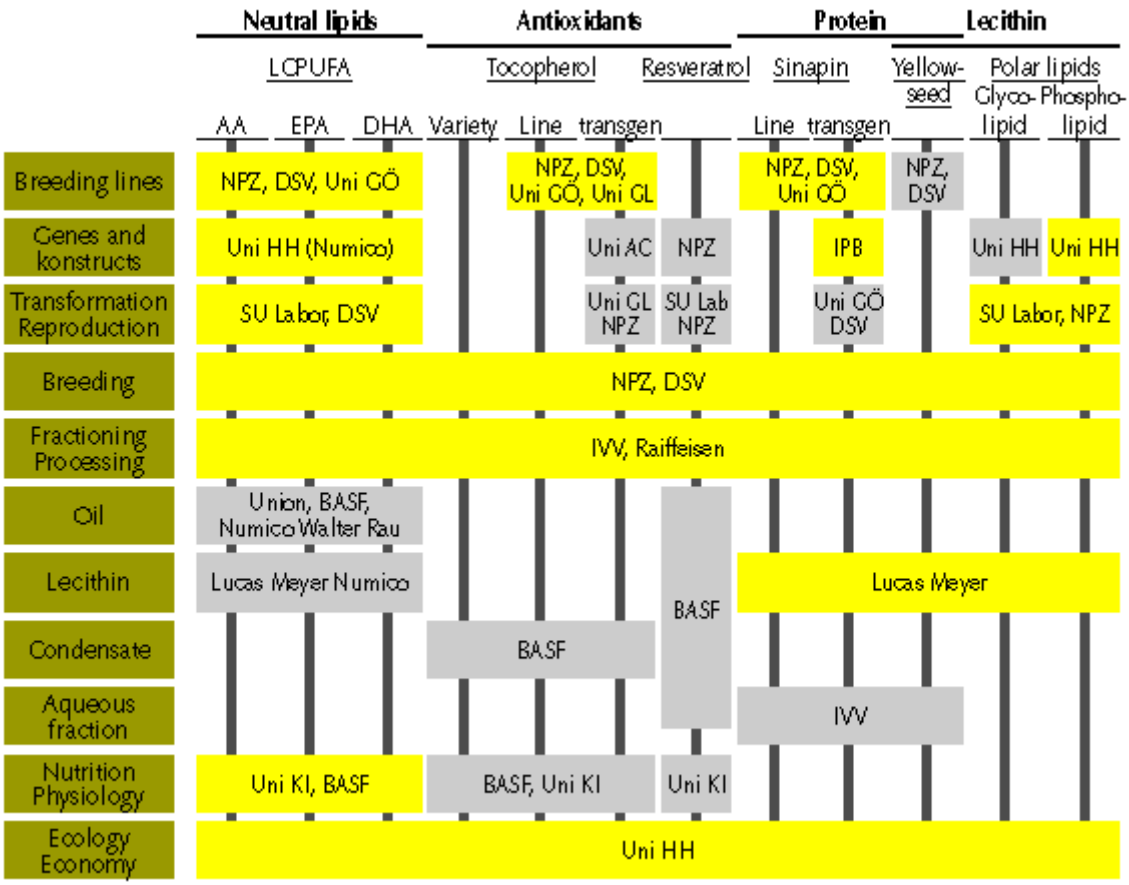


Figure 2. *Partners in NAPUS 20000.*

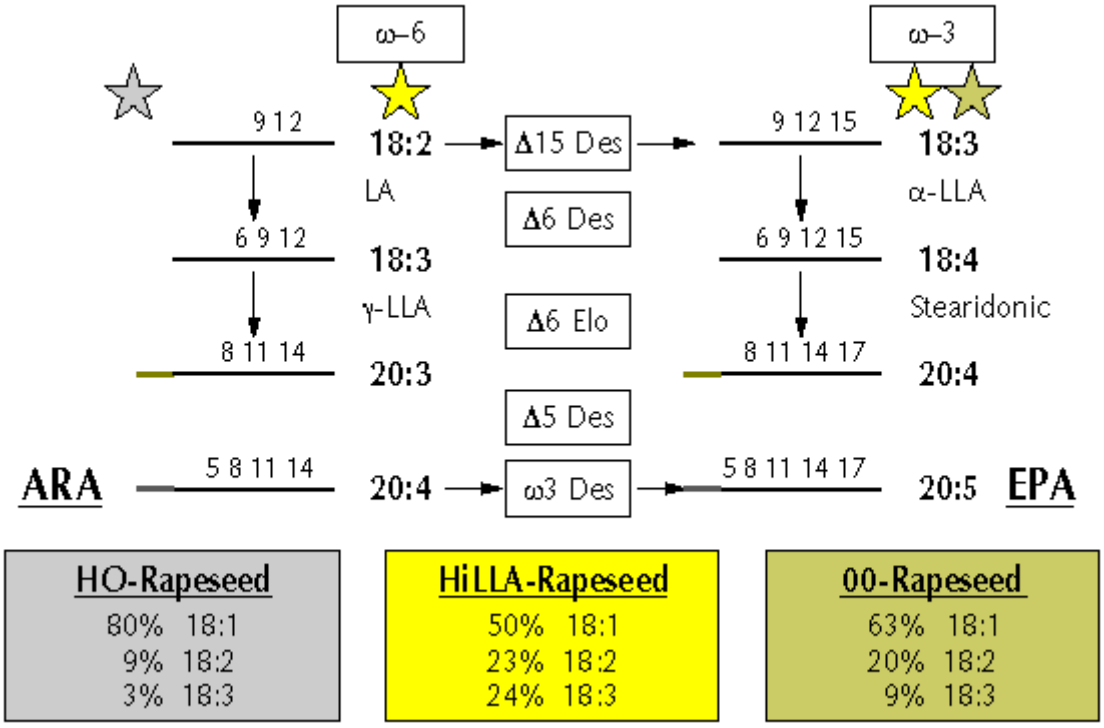


Figure 3. *Different basic plant material for the production of LCPUFAs in rapeseed oil.*