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Vorhabenbezeichnung: GABI-Kanada (CGAT): YelLowSin - Functional genomic approaches for the development of yellow-seeded, low-sinapine oilseed rape (Canola; <i>Brassica napus</i>) / Teilprojekt F	
Laufzeit des Vorhabens: 01.03.2006 bis 31.05.2009	

Schlussbericht

I. Kurze Darstellung zu

1. Aufgabenstellung

The general aim of the project is the development of tools including respective plant material, to enable the breeding of significantly improved rapeseed varieties by reducing the unfavorable components in the kernel. Indeed although rapeseed is a valuable source of vegetable protein - due to its amino acid composition of the protein, which is near to the optimal nutritional value -, the dietary fiber content, the dark colored tannins as well as bitter tasting sinapate esters (sinapine) still hamper its economical use in animal and human nutrition.

The improvement of digestibility of the rapeseed meal, which is partly correlated with a thinner seed coat (yellow seed phenotype) as well as lower fiber content within the seeds is a putative trait of interest. Furthermore the general nutrition value of the rapeseed meal can be raised up with a reduction of the kernel sinapine content. These traits should be preferably transferred to elite germplasm to be integrated into the rapeseed breeding process to deliver agronomical performing rapeseed varieties and satisfy a continually increasing demand on rapeseed protein for both feeds and foods.

NPZ is linked with three work packages and supports the work of scientific partners by delivery of results from following tasks:

I. Marker assisted breeding of yellow-seeded low-sinapine genotypes; The NPZ owned yellow seeded YS-NPZ germplasm was made available for a screening on low fiber and sinapine content. The material needed significant improvement to reach the agronomic level of actual breeding materials by crossing with elite germplasm. The actual breeding material was analyzed for sinapine content to set up an initial pool of lines for the development of low sinapine breeding material combined with yellow seeded, low fiber material.

II. A high-throughput NIRS assays for sinapine content in the kernel was to be developed and improved based on a comparison of sinapine profiles measured by NIRS and HPLC-analysis performed by IPB. Using these techniques, conventional oilseed rape breeding material had to be screened for naturally-occurring variation for sinapine content. Additionally, a set of substitution lines developed on a cross between a high-sinapine (Samourai) and a low-sinapine parent (Mansholts) was screened for sinapine content to characterize the plant material and eventually fine-map corresponding marker loci. Field trials and phenotypic screening of the substitution lines was performed on trail sites of NPZ, DSV and KWS to study environmental effects on sinapine content.

III. Generation and processing of an EMS mutant population for TILLING. Using an inbred line of yellow-seeded spring-type line (canola quality, owned by AAFC) a suitable TILLING population (based on a homogeneous yellow-seeded spring rapeseed) has been developed by NPZ in collaboration with UKI as part of an associated project funded by the Innovation Foundation of Schleswig-Holstein (ISH). TILLING assays for selected target genes from the sinapine pathway have been used for an extensive screening process for the desired mutations (UKI & NPZ). This was to be complemented by a phenotypic screening of the mutants.

2. Voraussetzungen, unter denen das Vorhaben durchgeführt wurde,

This subproject was part of the GABI consortium “YellLowSin: Functional genomic approaches for the development of yellow-seeded, low-sinapine oilseed rape / canola”. Within the overall goal of developing oilseed rape breeding lines with improved meal quality, the specific role of this subproject was to support breeding activities for low-fibre, low-phenolic winter oilseed rape. The YellLowSin project was one of two consortia funded within the BMBF program “Canada/Germany Agricultural-Genomics Team-building (CGAT)”, and was included as a co-funding consortium within the Canadian canola genomics project “Designing Oilseeds for Tomorrow’s Market” (DOTM). The work packages were closely aligned with relevant DOTM research groups.

Canada and Germany are two of the world’s leading producers of oilseed rape canola. The Canadian and German partners involved in this cooperation have considerable expertise in canola breeding and *B. napus* functional genomics research. Whereas Canadian breeding programs are strongly oriented towards spring-type canola varieties, winter-type oilseed rape cultivars are dominant in Germany. This makes cooperation with Canada an ideal opportunity to combine the resources and expertise of the two countries in canola breeding and genomics without conflicting commercial interests. The collaboration was based on a mutual exchange of plant materials and functional genomics resources (e.g. molecular markers, gene constructs, markers and gene expression data) and division of tasks (e.g. allelic screening, transformation, breeding, TILLING) with the Canadian partners to achieve common goals in a complementary and synergistic manner.

3. Planung und Ablauf des Vorhabens,

Workpackage 1

WP 1.3 Localization and fine mapping of QTL and development of markers for seed colour, fibre components and tannins including the validation using allelic tests of yellow seeded breeding lines (di-allele experiment)

Various available genetic resources known to include alleles responsible for a yellow seed color were crossed to dissect the digenic interaction for alleles segregating within this plant material. Segregating F2-seeds were produced by Partner UGI together with Partner DSV using also the NPZ source. The Segregation pattern of the trait for diverse crosses was evaluated on different locations in Germany. Segregating F2-Populations indicated different alleles for the seed color genes, thus identifying different sources of the alleles, at least for the YS-NPZ source.

Workpackage 2

WP 2.1 Pedigree breeding for stable yellow-seeded rapeseed

Based on the NPZ owned source of yellow seed color (NPZ-YS) new crosses were set up to

integrate the alleles for the trait into elite breeding material. For this purpose the original genotypes were crossed with winter and spring type lines. Subsequent selection for seed color was made to increase the stability of the trait within the plant material. It was intended to reach a level of yellow seed color stability which allows the use of the trait in commercial varieties.

WP 2.2 Implementation of suitable markers in marker-assisted breeding for yellow-seeded OSR

Markers for the main gene loci on chromosome N09 were developed and verified using existing mapping populations. A further unselected double haploid mapping population was built up to map and identify minor loci influencing the seed color. A seed color scoring was assessed in the greenhouse only. The population was sown for field evaluation in the season 09/10. Three further populations were preselected using the newly developed markers for the major gene. Based on the seed color scoring results from these populations, the reliability of the markers will be validated. This will be done after the seeds are harvested in 2010.

Workpackage 3

WP 3.1 Development of a high throughput NIRS-Assay for sinapine

Seed samples harvested from preselected plants from the breeding material were analyzed using NIRS and a parallel HPLC analysis. The resulting spectrum from the NIRS readout was used to set up a calibration, which at least in parts, reflects the sinapine content of the respective samples. The availability of a reliably working sinapine calibration enabled the company to select for interesting genotypes. Using this system the throughput was dramatically increased. However, improvement of the calibration still is mandatory, probably due to masking compounds within the seeds.

WP 3.2 Phenotypic evaluation: Generation of new vectors silencing new target genes (sinapate ester analysis and metabolite profiling)

The characterization of transgenic plants was the task of NPZ within this work package. Different constructs harboring genes encoding enzymes from the phenyl-propane pathway have been generated by IPB Halle and used for rapeseed transformation by partner SU-Bio (SU-Lab). Transgenic plants were then transferred to NPZ for phenotypic characterization and production of T2 seeds. Biochemical and genetic analyses of transgenic T2 seeds were subsequently performed at IPB.

Phenotypic analysis of the transgenic plants in the green house (using appropriate control plants) did not reveal any dramatic phenotype in plant growth. However, because of the high level of heterozygosity within the T1 generation, phenotyping of advanced transgenic generations with the desired seed traits will reveal, whether any unexpected phenotype can occur upon seed specific modulation of the phenylpropanoid pathway.

WP 3.4 Basic characterization of a set of Substitution-Lines

A set of 284 substitution lines has been made available for the project internal experiments. One of the parental lines from the initial cross was known to inherit a lower sinapine content (Mansholt's), while the other parent resembles a historic *Brassica napus* elite germplasm

(variety "Samourai"). The plant material was grown on small plots. From each plot selfed seeds from four plants were bulked and used for NIRS analysis. A set from the same seed bulk also has been sent to IPB-Halle for HPLC analysis. Data from the NIRS readouts indicated a slight reduction of sinapine below the content of the donor Mansholt's in some lines, but the majority of lines showed sinapine contents in the range of the recurrent parent or above.

WP3.5 Generation of an EMS mutant population for TILLING of sinapine biosynthesis key genes

A TILLING population of the final size N=3707 M2 plants was generated using the Canadian yellow seeded genotype YN01-429 (kindly provided by Dr. Rakow, AAFC Canada). Leaf material from single M2 plants was harvested for DNA extraction and plant development was scored during the whole growth period. Based on results from previous projects, putative candidate genes were identified to play a significant role within the synthesis of sinapine (phenylpropane pathway). A sequence analysis revealed a very high sequence homology between different copies and alleles of the target (*Brassica napus* UDP-glucose:sinapate glucosyltransferase (UGT84A9)), thus indicating problems for the development of suitable TILLING assays. The TILLING population was further analyzed using NIR spectroscopy to search for mutants showing reduced sinapine contents. Identified 18 mutants were subjected to further field characterization without being able to repeat the reduced sinapine contents when reanalyzing them using wet chemistry. M3 seed batches from all M2 lines releasing seeds have been stored at NPZ and are available upon request by partners.

4. wissenschaftlichem und technischem Stand, an den angeknüpft wurde, insbesondere

4.1 Development of plant material for genetic analysis and breeding of yellow-seeded winter oilseed rape

Based on previous projects and own breeding efforts, diverse plant materials were available, which have been used within the YeLowSin project. Different *B. napus* populations, segregating for seed colour and dietary fibre content, were developed in the GABI-GARS project (UGI, DSV, KWS, NPZ). The involved plant breeding companies DSV, KWS and NPZ developed different yellow-seeded plant materials within their own breeding programs. Further yellow-seeded lines of different origin are available (at partners NPZ and DSV).

4.2 Development of plant material for genetic analysis and breeding of low-sinapine oilseed rape

The development of a set of substitution lines has been continued by DSV, KWS and NPZ based on material developed at the University of Goettingen during the GABI-GARS project. The development of a suitable TILLING population (based on a homogeneous yellow-seeded spring oilseed rape line) was started by NPZ in collaboration with Partner UKI as part of a project funded by the Innovation Foundation of Schleswig-Holstein. Further conventional oilseed rape breeding material that segregates for sinapine content (5-10 mg/g seed), was available within private breeding resources. Transgenic lines (low sinapine) are available in spring type rapeseed based on preliminary work by NPZ, SU-BIO, DSV and IPB on the German side through activities going back to the project NAPUS2000 and by AAFC-MG and PBI on the Canadian side.

4.3 Development of resources for genetic mapping and transformation

A large set of private and public *Brassica* SSR markers was available at beginning of the project together with detailed mapping data for the markers. A large number of gene constructs were available at IPB for the generation of transgenic *B. napus* lines with alterations in key

genes for sinapine biosynthesis. These constructs have been in part produced during the NAPUS2000 project together with NPZ. The BAC library (cv. 'Express') was also developed during GABI-GARS. Controlled greenhouse experimental conditions for testing of genetically modified material (S1 facilities) were established by NPZ.

4.4 Optimisation of TILLING parameters and creation of a *B. napus* EMS-mutant population

The parameters for mutagenic treatment of *B. napus* have been established at UKI in tight cooperation with a NPZ coworker. DNA extraction on a 96-well format was optimized for robot-based extraction, normalization and pooling of DNA. The reverse genetic tool for systematic discovery of allelic series of EMS-induced point mutations was completely established. For rapeseed, a TILLING population (based on a homogeneous yellow-seeded spring oilseed rape line) is being developed as a core EMS mutant collection of 5000 selfed M1 plants in collaboration between NPZ and UKI as part of a project funded by the Innovation Foundation of Schleswig-Holstein.

4.4.6 Genetics and biochemistry of sinapate ester biosynthesis

Technological basis for this work package relies on previous activities done in the framework of NAPUS 2000. The isolation and characterisation of target genes, was mainly done by Partner IPB Halle (Milkowski et al., 2000a and 2000b, 2004). First analysis of transgenic activities for the gene *BnSGT1* were described by Hüsken et al. (2005).

- *Angabe der verwendeten Fachliteratur sowie der benutzten Informations- und Dokumentationsdienste,*

Hüsken A, Baumert A, Strack D, Becker HC, Möllers C, Milkowski C (2005) Reduction of sinapateester content in transgenic oilseed rape (*Brassica napus*) by dsRNAi-based suppression of *BnSGT1* gene expression. *Molecular Breeding* 16: 127–138.

Leckband G., Frauen M., Friedt W. (2002) NAPUS(2000). Rapeseed (*Brassica napus*) breeding for improved human nutrition. *Food Res Int* 35: 273-278.

Milkowski C, Baumert A, Schmidt D, Nehlin L, Strack D (2004) Molecular regulation of sinapate ester metabolism in *Brassica napus*: expression of genes, properties of the encoded proteins and correlation of enzyme activities with metabolite accumulation. *Plant J.* 38: 80-92.

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

Milkowski C, Baumert A, Strack D (2000b) Identification of four Arabidopsis genes encoding hydroxycinnamate glucosyltransferases. *FEBS Lett* 486: 183-184.

5. Zusammenarbeit mit anderen Stellen.

The cooperation within the project included scientific institutions and breeding companies from Germany. Breeding groups from Canadian Universities were also involved besides the molecular biology group at NRC-PBI in Saskatoon. Members of the YelLowSin consortium attended all meetings of the DOTM project, and vice versa. Project data and results including plant material were fully accessible from Canadian and German sides under an international cooperation agreement and a joint material transfer agreement.