

Public-private interaction for research related to oilseed rape breeding in Germany

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ABSTRACT

Research related to breeding for improvement of winter oilseed rape in Germany has thrived in the past decade in an environment in which high R&D investment from breeders, particularly those producing high-revenue hybrid seed, has encouraged significant governmental investment into crop genomics, phenomics and innovation-based breeding research. Around half of the staff currently involved in oilseed rape research in our group are funded within public-private consortia or bilateral collaborations with breeding companies. The most commonly applied model for public-private research funding favours constellations with 100% funding of the non-profit institutions and a 50% funding contribution to the costs of the commercial partners. In general the investment by the participating companies should make up 25-30% of the overall project costs. Depending on their size and resources, breeding companies tend to contribute "in kind" – either through the generation of suitable plant populations, phenotyping of relevant traits in field trials or provision of molecular marker data by affiliated service labs. In return they gain – in the context of a contractual consortium agreement – preferential or exclusive access to novel plant material, genetic markers, DNA sequence data, novel phenotyping tools and/or genomic resources that are generated by the participating scientists. Industry-driven research appeals to funding agencies due to its tendency to promote technological progress within industry and agriculture. Participating breeding companies benefit not only from their direct access to research results that most would generally not be able to achieve with their own resources, but also from the "subsidisation" of their R&D investments. Academic partners willing to perform more applied research benefit from increased access to grant funding, which tends to have spinoff effects into improved public funding for more basic research (with potentially better publication potential). Recent and current research consortia in which our group has worked together with the oilseed rape breeding industry include projects for genomics and innovation-based breeding towards improvement of seed meal quality, drought tolerance, seedling vigour, disease resistance, oil content, oil quality and seed yield. This paper presents selected examples of recent and current research activities and their results, describing the context of different interactions with commercial breeders within public-private oilseed rape research consortia.

Key words: Rapeseed, Germany, genomics, breeding, research

INTRODUCTION

Winter oilseed rape is the leading oilseed crop in Germany, with a current production area of around 1.5 million ha and average yields of more than 4 t/ha leading to a total seed production of over 6 million tonnes per annum. More than 50% of the production today derives from restored F1 hybrid varieties, and the proportion of hybrids in the seed market is increasing steadily as the development of new parental pools continues to increase yields and yield stability in comparison with open-pollinated varieties. The high seed sales from F1 hybrids provide a high level of revenue for successful breeders, who in turn are able to invest more heavily in research and development than is the case for less lucrative crops. Cultivar development in oilseed rape and other major crops in Germany is today performed almost exclusively by commercial breeding companies, as universities and public research institutions no longer have the financial resources and infrastructure to compete on the seed market. On the other hand, the growing demand for high-performing cultivars of high-value hybrid crops like oilseed rape, sugar beet and maize has enabled a considerable number of mid-sized seed

companies to remain competitive with the larger multinational enterprises that dominate the seed market in other parts of the world. On the other hand, this ability to compete relies heavily on investments in external R&D partnerships, since only the largest companies are in a position to cover the full spectrum of research with their own expertise and facilities. Small and mid-sized enterprises therefore form strategic research alliances with relevant scientific institutions, in consortia that include one or more cooperating breeding companies and one or more academic partners.

Such consortia are eligible to compete for funding in appropriate calls for applied, public-private research funded by the relevant federal ministries. By requiring co-funding from industry partners as a general prerequisite for their research calls, the funding bodies preferentially inject money into industry-driven research which per definition should be more likely to result in short-term and/or long-term economic benefits for the specific commercial partners and for the agricultural industry in general. Participating breeding companies benefit not only from their direct access to research results that they may not be able to achieve with only their own resources, but also from the financial contribution to their R&D investments from the funding agencies. Academic partners willing to perform more applied research benefit from increased access to grant funding, which tends to have spinoff effects into improved public funding for more basic research. The mandate of the research community to publish scientific results from public-private research is generally secured by a consortia agreement that sets out a framework for distribution, exploitation and publication of plant materials, intellectual property and data from the cooperative research. This model has been followed in numerous calls in the field of crop breeding and genomics over the past two decades, with funding being provided particularly from the federal Ministry of Education and Research (BMBF), the Ministry of Nutrition, Agriculture and Consumer Affairs (BMELV) and the Federal Agency for Renewable Resources (FNR). The BMBF has sponsored a number of large, long-running programs with a strong participation from the plant breeding industry, including the national plant genomics research platform "GABI", the European Plant-KBBE ("Knowledge-based Bioeconomy") program and the new "Biotechnology for the Future" platform. The BMELV funds numerous multidisciplinary, industry-driven "Innovation" programs specifically designed to transfer scientific knowledge into industrial products. In the crop science field the most recent BMELV calls are funding innovations in breeding for climate change focussed on bioenergy crops, a focus also supported by a recent FNR call.

One important body that is heavily involved in sustaining the momentum of crop breeding research in Germany is the Society for the Promotion of Private Plant Breeding (GFP e.V.). The GFP is one of three organisations that work together in Bonn under the auspices of the Federation of German Plant Breeders (BDP), an umbrella organisation for plant breeding companies that represents their interests with active participation in bodies who determine priorities for agricultural research and politics on a national and European Union level. Together with the licensing and patenting agency GVS, who provide member companies with assistance in IP claims and disputes, and the seed certification agency STV, who represent breeders' rights in obtaining revenue for certified seed, the GFP has a representative function for participating breeders with respect to various political, economic and legal issues. A key function of the GFP is coordination of research activities between the scientific and commercial communities: Breeders participate in sections dealing with specific crops or groups of crops (e.g. cereals, maize, sugarbeet, oil and protein plants) and communicate research priorities that they identify as worthy of particular attention either in specific crops or in breeding in general. Priorities set by the breeding industry are subsequently communicated by the GFP to political bodies involved in development of new public-private research calls, on the one hand, and on the other hand to relevant members of the scientific community who have the expertise required by the breeders to achieve their stated goals. Together with the GFP and the breeders the scientists then formulate research proposals to address the needs of the breeders. This tends to result in funding for applied research programs with a high practical relevance to current problems in breeding and agriculture, and gives scientists who are prepared to perform more applied research very good opportunities for funding and a long-term alignment with cooperating industry partners. In some cases the participating breeders share plant materials or selection tools (e.g. markers) developed by the scientific groups for use in their own breeding programs, in other cases the breeders utilise their own proprietary germplasm and take advantage of

technologies (e.g. DNA sequencing, metabolite profiling, deep phenotyping, etc.) that they may not otherwise have the resources to apply within their normal breeding programs.

Table 1. Major German oilseed rape research consortia involving IPZ-JLU over the past ten years.

Funding agency	Project / Theme	Duration (years)	Number of partners		JLU-IPZ staff funded ²	Coordination
			Commercial companies	Scientific institutions		
BMBF	NAPUS 2000: Next-generation GM traits	5	5	7	1 PD, 1 TA	Industry partner + JLU
BMBF	GABI-GARS: Genome analysis in rapeseed	3	5	3	1 PD, 1 PhD, 1 TA	IPZ-JLU
BMBF	GABI-BRIDGE: Allelic diversity in candidate genes	3	5	3	1 PhD, 1/2 TA	Univ. Göttingen + industry partner
BMBF	GABI-YelLowSin: Functional genomics for meal quality	3	4	3	2 PhD, 1 TA	IPZ-JLU
FNR	Verticillium disease: Development of resistant germplasm and selection markers	6	4	3	1 PhD, 1 TA	GFP
BMELV	Verticillium resistance: Marker-assisted breeding and resistance gene cloning	3	8	2	1 PD, 1 TA	GFP
BMBF	KBBE-CONVIGOUR: Functional genomics of seed vigour	3	5	5	1 PhD, 1 TA	Industry partner
BMELV	Innovations in breeding for climate change: Selection tools for drought tolerance	3	7	2	1 PhD, 1 TA	GFP
BMBF	PreBreed Yield: Precision breeding for yield gain	3	7	7	1 PD	Industry partner

¹ BMBF: Federal Ministry of Education and Research; BMELV: Federal Ministry of Nutrition, Agriculture and Consumer Protection; FNR: Agency for Renewable Resources.

² PhD: PhD student on half postdoc salary; TA: Full-time technical assistant; PD = postdoc

The Department of Plant Breeding at Justus Liebig University in Giessen (IPZ-JLU) has in the past two decades become one of the leading research partners for German companies involved in breeding of winter oilseed rape. Over the past decades we have participated in numerous public-private oilseed rape research consortia as scientific partner and/or coordinator (see Table 1 for details). Currently we are employing 12 PhD students, two postdoctoral scientists and a number of technical staff in projects on oilseed rape breeding, genomics and phenomics. Around half of those staff are currently funded within the framework of public-private

research consortia or bilateral cooperations with the plant breeding industry. In the following we provide three examples of different research consortia with specific breeding goals, along with an overview of the consortium structure, the contributions of the respective scientific and commercial partners and some of the most important results (in regard to commercial breeding aims) that have been obtained or are expected in the coming years.

Example 1: "YeLLowSin" – Functional genomics approaches for breeding of yellow-seed, low-sinapine oilseed rape/canola

The proportions of oilseed rape/canola meal that can be used in livestock feed mixtures are limited by the presence of major anti-nutritive compounds. Especially relevant in this regard are dietary fibre, dark-coloured tannins and bitter-tasting sinapate esters. Yellow seeds have been of particular interest for oilseed rape breeding because their thinner seed coat is associated with reduced quantities of anti-nutritive fibre and phenolic substances. However, selection for the yellow-seeded phenotype is difficult due to strong environmental effects. Measurement of fibre and phenolic fractions is time-consuming and difficult to automate, and furthermore involves destructive methods that are not suitable for selection in the breeding process. High-throughput, non-destructive measurement techniques for anti-nutritive compounds, for example, near-infrared reflectance spectroscopy (NIRS) assays or gene-linked selection markers for important trait loci, would represent a significant advance for germplasm screening, breeding and trait-genotype analysis. The YeLLowSin consortium, funded from 2006-2010, involved three academic institutions in Germany and three oilseed rape breeding companies with an overall goal of developing oilseed rape breeding lines with improved meal quality. The YeLLowSin consortium was funded within the BMBF program "Canada/Germany Agricultural-Genomics Team-building (CGAT)", and was included as a co-funding consortium aligned with the Canadian canola genomics project "Designing Oilseeds for Tomorrow's Market" (DOTM). The work of the German partners was closely aligned with relevant DOTM research groups.

Canada and Germany are two of the world's leading producers of oilseed rape/canola, and 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 same arguments might be used in future to promote German-Australian canola research. 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. Our major role in the consortium, besides responsibility for the scientific coordination, was the development and application of high-throughput techniques for identification of low-fibre, low-tannin oilseed rape lines. The specific aims were:

- 1) Establishment of reference measurement techniques and development of high-throughput NIRS assays for dietary fibre components;
- 2) Quantification and characterisation of seed phenolic acids and condensed tannin fractions;
- 3) Mapping of QTL and development of gene-linked markers for seed colour, fibre components and phenolic compounds.

"YeLLowSin" – Major accomplishments

We established chemical screening methods for key fibre and phenolic compounds and used these to generate near-infrared reflectance spectrometry (NIRS) assays for non-destructive selection of low-fibre breeding lines (Table 3; Wittkop *et al.* 2009). The participating breeding companies now use these NIRS calibrations as proprietary IP in their commercial breeding programs, enabling a high-throughput selection for fibre content in combination with low glucosinolate content, high oil content and fatty acid composition. Furthermore, we use the fibre NIRS calibrations to screen experimental populations for QTL mapping and association genetics analyses. By screening large *B. napus* diversity sets and a number of bi-parental mapping populations grown in multiple years and environments we identified important

quantitative trait loci (QTL) contributing to anti-nutritional fibre and phenolic compounds. For a number of QTL an association with genes involved in phenylpropanoid biosynthesis could be established by comparative mapping of relevant candidate genes. Synteny analysis of the major QTL for seed colour and fibre content enabled the delineation of a small genome sequence region that contains a small number of interesting candidate genes for reduction of seed coat cell wall compounds. Recently we have come very close to confirming a key lignin biosynthesis gene as the major functional candidate gene at this QTL for reduction of seed coat lignin and corresponding improvement in seed meal quality (Liu *et al.* 2011). The major finding of this study for breeders was that dark-seeded *B. napus* contains considerable genetic variation for seed coat lignin content. This enabled us to generate progenies from crosses among high-performing, elite dark-seeded varieties with high-oil and low fibre content, respectively. The NIRS selection tools and low-fibre germplasm that we developed in this project, together with the participating breeders represent a significant advance towards an overall improvement of seed quality for oil and meal production.

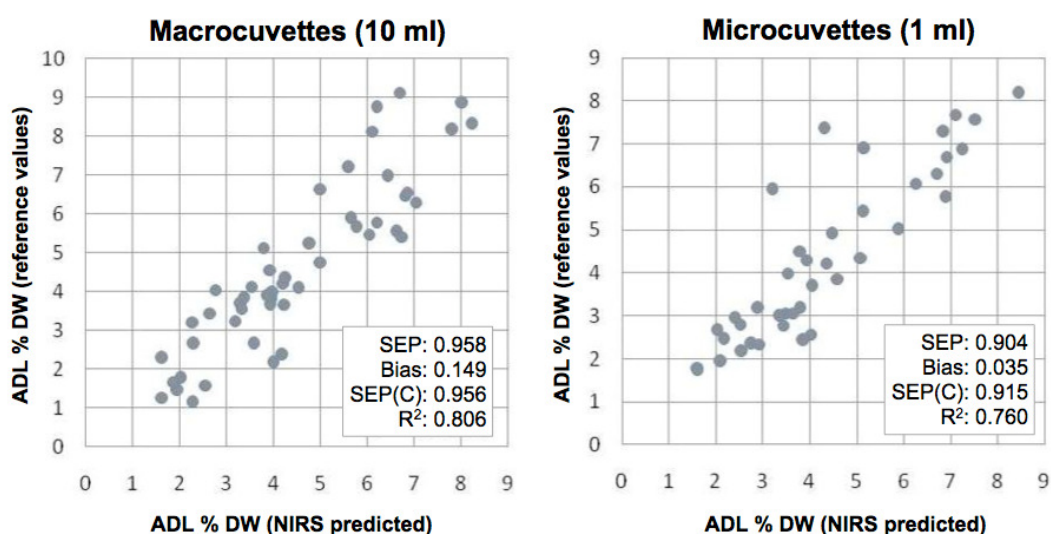


Fig. 1. Correlation and cross-validation statistics of new NIRS calibrations for non-destructive high-throughput measurement of antinutritive acid detergent lignin (ADL) in seed samples from winter oilseed rape measured in macrocuvettes (seed volume 10ml) and microcuvettes (seed volume 1 ml). DW: seed dry weight; SEC: standard error of calibration; R²: correlation coefficient; SECV: standard error of cross validation, R²CV: correlation coefficient of cross validation.

Example 2: Development of resistance against *Verticillium longisporum* and tools for marker-assisted introgression into elite winter oilseed rape germplasm

Verticillium disease caused by the vascular fungal pathogen *Verticillium longisporum* is one of the major diseases of oilseed rape in Europe. Current cultivars possess only a low level of resistance against *V. longisporum*, meaning that heavy infection can cause major yield losses. *Verticillium* is a soil-based pathogen that infects plants via the roots and colonises the xylem, meaning that control with fungicides is not possible. Due to the long persistence of microsclerotia in the soil for more than a decade, the only effective control measure is through development of resistant cultivars. Unfortunately no effective resistance is available in current European winter oilseed rape breeding materials, with the best-performing varieties showing little more than a somewhat elevated tolerance to *V. longisporum* infection. Even though disease symptoms are not always visible until shortly before or after harvesting, disease within the xylem vessels in the hypocotyl and stem can have a particularly strong negative effect on seed oil content and seed yield. Due to the complex nature of the disease and the quantitative, environmentally sensitive expression of disease symptoms, selection for resistance is extremely time-consuming and difficult, meaning that breeders previously had no means to select lines

with improved resistance. Due to the high priority of this disease in Europe the German oilseed rape breeders initiated research to develop plant materials and screening tools for effective breeding of high-performing winter oilseed rape materials with elevated levels of *V. longisporum* resistance.

This consortium involves nine breeding companies, who are members of the GFP section "Oil and Protein Crops", along with two scientific partners. Our group was responsible for identifying resistance donors in gene bank materials from *Brassica oleracea* and *Brassica rapa*, and for generating resynthesised *B. napus* lines with high levels of resistance derived from geographically and genetically diverse resistance donors. Subsequently we performed genetic mapping studies to identify molecular markers linked to important QTL involved in resistance. These are now being applied in marker-assisted backcrossing programs of the participating breeders to transfer the responsible resistance genes into elite winter oilseed rape breeding lines. The other scientific partner, the Institute for Phytopathology at Georg August University, Göttingen, is responsible for resistance screening under field and greenhouse conditions using experimental cross populations along with breeding materials supplied by the participating breeders. Both scientific groups are working on elucidation of the potential resistance mechanisms of the best-performing germplasm. In the most recent round of funding (2011-2014) we will concentrate particularly on the influence of *Vorticillium* disease and resistance on yield and oil content in the face of drought stress, a highly important exacerbating factor for diseases that cause blockages of the vascular system.

Verticillium resistance – Major accomplishments

Within this project we were able to generate a number of novel resynthesised *B. napus* lines carrying significant resistance against *V. longisporum* from very different genetic backgrounds (Figure 2a). The most effective resistances were found in C-genome donors from different *B. oleracea* subspecies. Interestingly, QTL mapping and comparative marker analyses in progenies from different resistance sources revealed that most of the resistance sources carry the same major resistance locus on the C genome chromosome C05, which contributes a quantitative resistance in association with contributing QTL on chromosome C01 and other minor QTL (Figure 2b). Metabolite profiling in hypocotyl samples from resistant and non-resistant progenies with and without inoculation uncovered a potential association of the resistance to concentrations of specific soluble phenolic compounds.

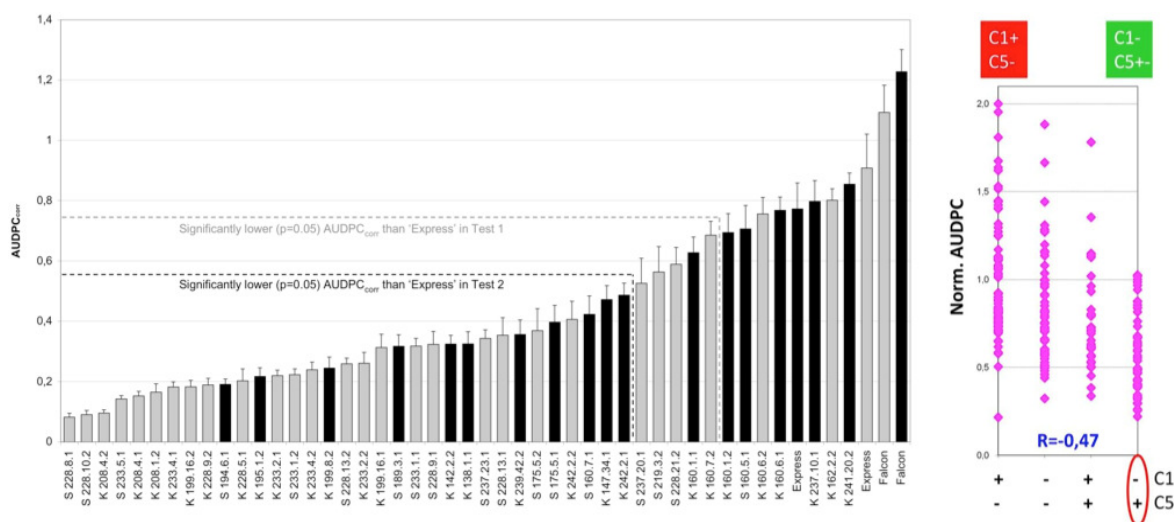


Fig. 2. (a) *Verticillium longisporum* disease scores of 45 resynthesised (RS) rapeseed lines (prefix 'K' and 'S'), along with the reference rapeseed cultivars 'Express' and 'Falcon', measured by (normalised area under the disease progress curve; AUDPC_{corr}). The light and dark bars represent results from two independent resistance experiments (test 1 and test 2) along with the respective controls from each of the two tests. A number of RS lines show significant resistance compared to the moderately tolerant control, 'Express'. (b) Marker-assisted selection of

progenies from crosses with resistant RS lines revealed considerable reduction of AUDPC in lines containing markers linked to a major QTL on chromosome C05 from the RS resistance donor (+) in combination with markers for a contributing QTL on from the tolerant parent 'Express' (-).

Furthermore, a key gene involved in phenylpropanoid biosynthesis was localised in the QTL on chromosome C01, with positive alleles derived from a moderately tolerant, conventional winter oilseed rape variety. The markers identified in this study have been shown to be effective for marker-assisted combination of the most important quantitative resistance loci in elite germplasm. The most effective QTL-derived markers are now being implemented into commercial breeding programs to combine complementary resistance alleles into high-performing varieties.

Example 3: PreBreed Yield: Resources for nested association mapping and genomic selection

To date plant breeders and genomics researchers have failed to develop effective molecular selection tools for systematic identification of breeding materials with improved yield potential. The latest advances in whole-genome profiling using next-generation DNA sequencing and high-density SNP arrays raise hopes that genomic selection for highly complex traits – including yield – may become feasible in future. Before genomic selection for yield and other complex traits becomes possible, however, it is necessary to generate suitable plant populations harbouring all available novel and useful genetic diversity for relevant yield-related traits, along with comprehensive collections of genomic markers associated with genome-wide loci influencing relevant traits. Obtaining high-density genome-wide SNP information is relatively simple with the latest technologies, however a major and very expensive obstacle to obtaining trait-relevant genomic data is the necessity for extensive phenotype data for the test populations.

In order to achieve these goals a large new consortium has recently been initiated in Germany to develop a large, immortal, genetically diverse "nested association mapping" (NAM) population for winter oilseed rape. Furthermore we intend to genotype this population using a high-density SNP array, in combination with genomic sequence data from the founder lines, and to utilise the NAM population over the coming decades for phenotypic characterisation in classical field evaluations and with emerging phenomics technologies. The expected result is unique and broad collection of novel genetic diversity for breeding, together with the necessary genomics technologies, sequence data and phenotypic information for exploitation of that diversity in commercial breeding programs. The PreBreed Yield consortium, which will be funded for three years from autumn 2011, comprises seven industrial partners encompassing most of the major oilseed rape breeding companies in Germany, along with partners from seven scientific institutions covering all aspects from conventional and genomics-based breeding, phenotyping and phenomics through to biostatistics, database management and bioinformatics.

One commercial partner has assumed overall responsibility for the project coordination, while five others are together generating the NAM population, comprising a total of 2500 doubled-haploid (DH) or recombinant inbred lines (RILs). The last commercial partner is responsible for genomic resequencing of the 51 genetically diverse parental lines of the NAM population. Depending on their area of expertise the scientific partners are involved in genotyping, sequence analysis, phenotyping, bioinformatics, database management or association mapping, respectively.

The PreBreed Yield project plan

A large nested mapping population comprising around 2500 NAM lines is currently being generated from crosses that were made between 50 genetically diverse founder accessions and an adapted, high-oil, high-yielding DH line derived from a cross between two current elite Germany winter oilseed rape varieties. To maximise the genetic diversity of the NAM population the founder lines include 20 resynthesised *B. napus* accessions of genetically divergent origin, along with 30 genetically diverse *B. napus* inbred lines that were selected based on DNA

sequence data from conserved genes to cover as broadly as possible the genetic diversity present in the species. Crossing with a single elite parent generates interrelated segregating populations that on the one hand greatly increase the adaptation of the materials, an important consideration when planning field evaluations where yield and other complex traits need to be assessed. On the other hand the generation of segregating subpopulations from each founder line increases the frequency of rare alleles derived from only one or a few parental accessions. This considerably raises the power of detection for rare alleles with positive effects on traits of interest. Genome-wide SNPs linked to such rare alleles will enable implementation of this interesting novel germplasm in breeding for yield and other complex traits via genomic selection.

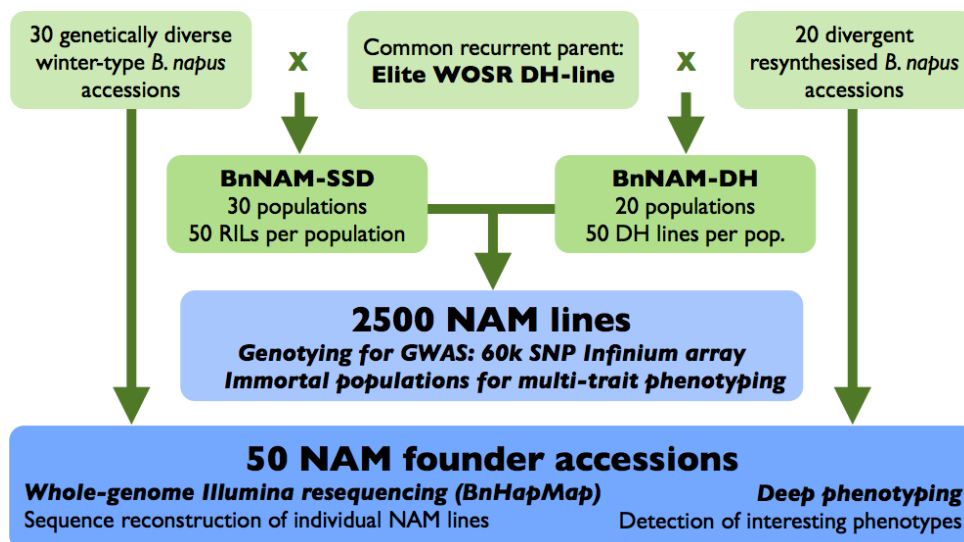


Fig. 3. Schematic overview of the planned population development, genotyping and phenotyping for nested association mapping and genomic selection within the PreBreed-Yield consortium.

From each of the 30 natural *B. napus* founder lines we are generating 100 DH lines each by single-seed descent in the greenhouse. The intention is to randomly select 50 DH lines per cross for the final NAM population. The 20 F₁s from the crosses with the RS *B. napus* lines are being backcrossed once with the adapted elite parent to increase the adaption of the materials for subsequent field evaluations. The BC₁ will be used to generate a minimum of 50 BC₁F₄ RILs per founder accession by single-seed descent in the greenhouse. The BnNAM-DH lines are expected to be completed in 2012 and will be genotyped with over 50,000 single-locus SNP markers using a new public *B. napus* Infinium SNP array being generated by a multinational *B. napus* SNP consortium. The BnNAM-SSD lines are expected to be completed and genotyped one year later. To extrapolate the whole-genome sequences of all NAM lines we will perform genomic resequencing of all 51 founder lines, making it possible via a haplotype map to "fill in" the sequence in every interval between two neighbouring SNP markers derived from the same parent. This will give us the basis to extrapolate from trait-linked markers identified in the association analyses to DNA sequence variation in positional candidate genes within the NAM germplasm.

Phenomics facilities of the scientific partners will be used for detailed phenotyping of numerous complex traits in the exotic founder lines to identify potentially interesting variation e.g. for oil content, seed quality, nutrient efficiency, biotic or abiotic stress resistances, etc. Furthermore, an initial trial to identify genome-wide markers associated with yield and heterosis will be performed by generating F₁ hybrids from a subset of 500 BnNAM-DH lines through controlled crosses with a male-sterile tester. The F₁ hybrids will be tested by the participating

breeders in multi-location field trials, giving the first data for genome-wide markers related to yield in this new germplasm, within the three-year funding period. Further analysis of yield and other complex traits, including detailed field-based phenotyping and high-throughput phenomic analysis of the entire NAM population, will continue in followup projects based on the immortal populations and data generated by the PreBreed Yield consortium. The ultimate result will be a repository of genome-wide marker data that will serve as a basis for utilisation of these novel NAM populations and genomic data in commercial breeding in the decades to come.

CONCLUSION

Public-private research funding is a valuable organ sustaining crop breeding research in Germany, and oilseed rape has been one of the major crops to benefit substantially from industry-driven research during the past two decades. On the one hand the availability of public research funding for industry partners enables even small and mid-sized enterprises to finance and perform cooperative research that may otherwise be inaccessible for them due to budget or infrastructure constraints. On the other hand strong links are fostered between the scientific and commercial breeding communities, encouraging exchange of innovative ideas, plant materials and novel breeding technologies. Scientific groups benefit from increased access to third-party research funding, the proviso being that they should focus on applied research with potential commercial benefits for the participating companies. From a scientific perspective it is often more difficult for scientists involved in applied research on crop plants to achieve high-impact publications in top-ranking journals, but a general trend is that scientific groups with a strong record of industry-derived funding are often also successful in transferring this momentum into successful funding for more fundamental research – with potentially more opportunities for higher-level publications.

One underestimated benefit of the partnership between leading crop research groups and the breeding industry involved in their particular crops of interest lies in the close ties that early-stage researchers are able to form with the breeding industry. This gives particularly doctoral graduates a notable advantage with regard to subsequent employment prospects in the commercial sector. Quite commonly PhD students who are involved in cooperative projects with breeding companies will be approached by one or more participating breeders with potential job offers before they themselves begin to actively search for employment. This in turn tends to further strengthen the ties between industry and academic research on a bilateral and consortia level.

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