

Breeding Strategies to Improve Nitrogen Use Efficiency in Oilseed Rape

J. Keurentjes, N. Erol-Oztolan, F. Breuer, A. Gertz, S. Miersch,
H.C.Becker



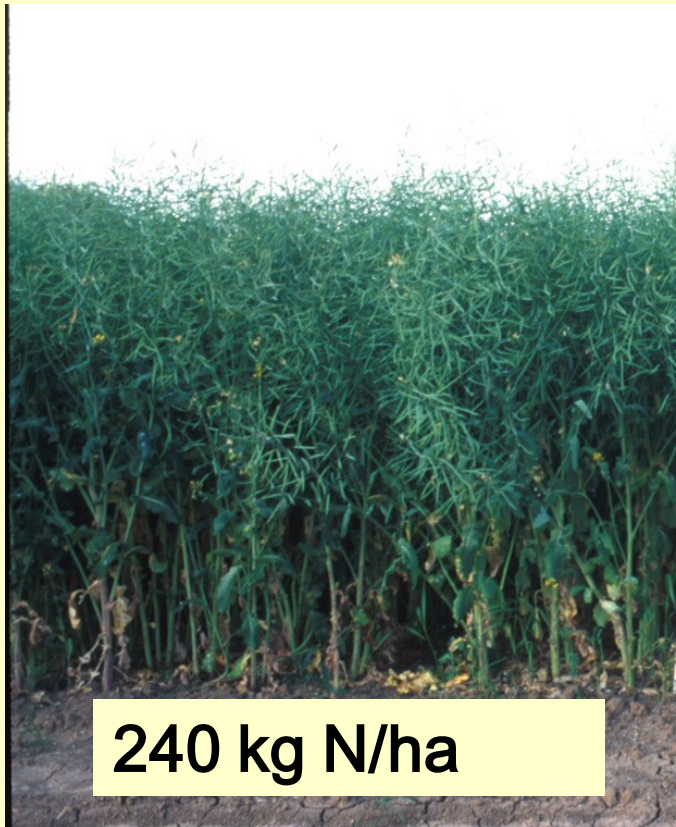
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- Introduction
 - The problem
 - The NUE-CROPS research approach
- Arabidopsis
- Oilseed rape
- Conclusions and Outlook

The Problem

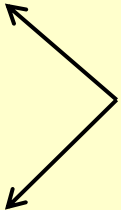
Oilseed rape reacts susceptible to reduced N supply



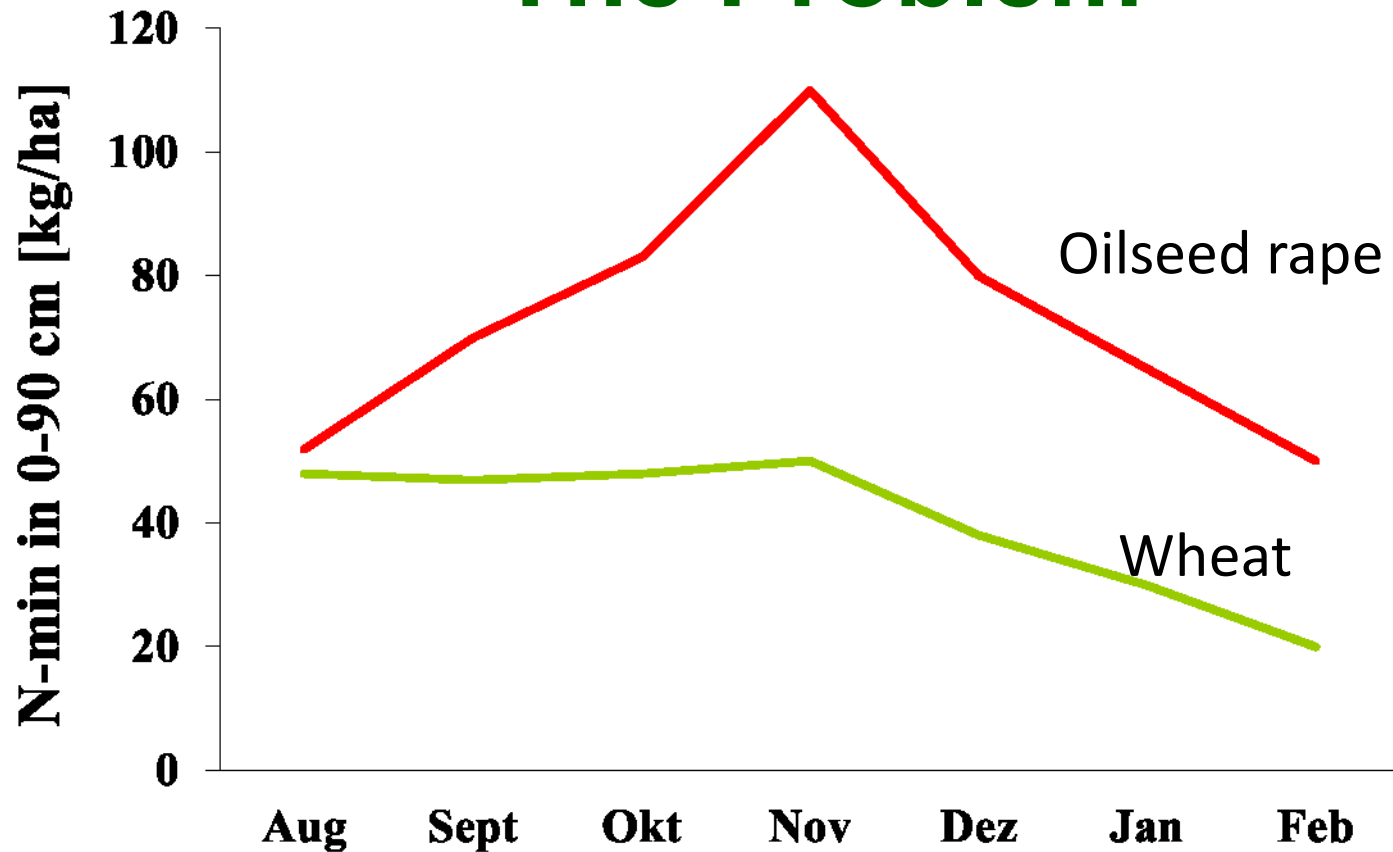
The Problem

- Oilseed rape is traditionally produced with high N-fertilizer inputs
- Oilseed rape is efficient in N uptake
- The N amount in harvested seeds is smaller than the N amount fertilized
- This may result in significant levels of nitrate leaching after harvest
- It is necessary to reduce the N surplus

Example

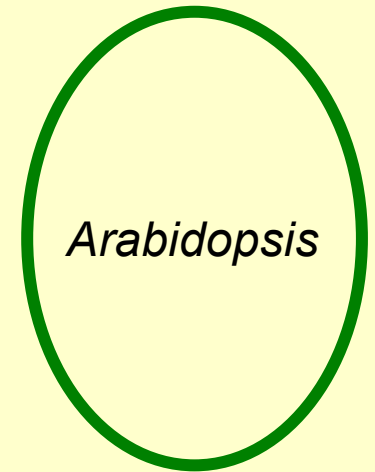
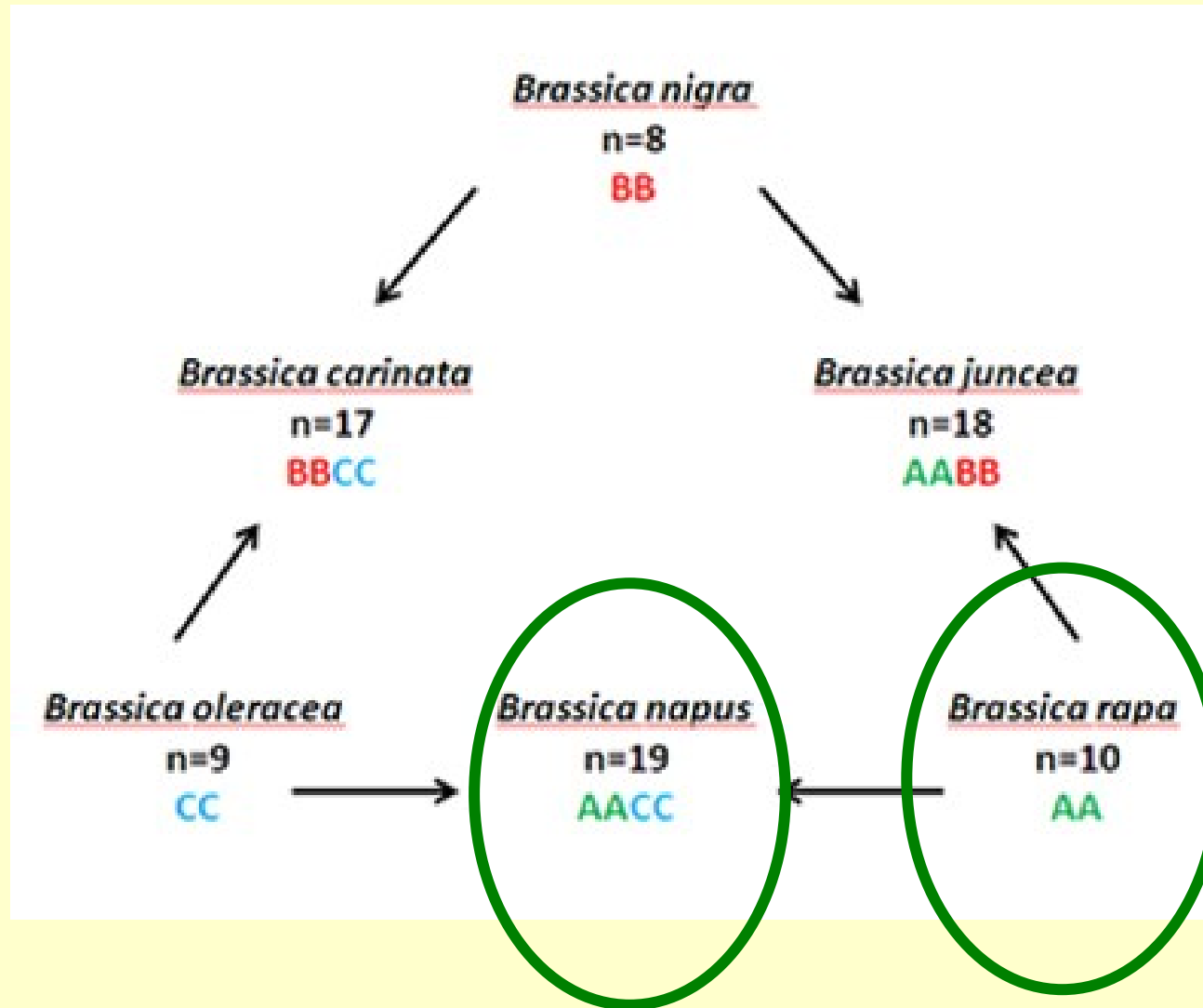
N Fertilization	180 kg/ha	 + 70 kg/ha
N in total plant biomass	330 kg/ha	
N in harvested seed	110 kg/ha	

The Problem



N-min after harvest of wheat and oilseed rape
(2 years, Höckelheim, LK Hannover)

The NUE-CROPS approach



Oilseed rape

The NUE-CROPS approach

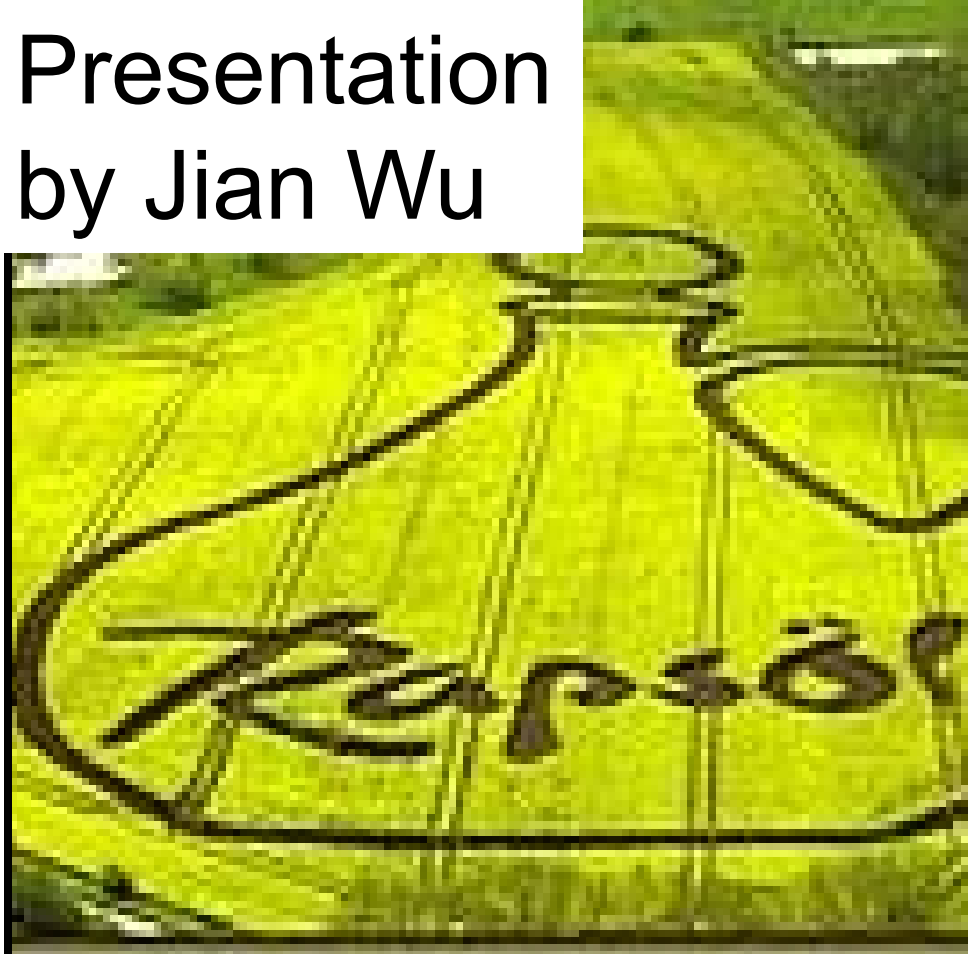


A. thaliana



B. rapa

Presentation
by Jian Wu



Oilseed rape (*B. napus*)

WP 3.1: Genetic Analysis of Nitrogen Use Efficiency in *Arabidopsis thaliana*

Nihal Öztolan Erol, Joost Keurentjes



WAGENINGEN UNIVERSITY
WAGENINGEN UR



As a Model Organism *Arabidopsis thaliana*

- Dicot used as model organism in many studies
- 5 chromosomes, 120 mb genome and ~30,000 genes
- Brassicaceae family – synteny
- Self fertilization
- World-wide natural variation



Materials and Methods

- **Plant Material:**
 - HapMap population (353 accessions)
- **Growth Conditions:**
 - On rockwool bloks, covered to prevent algae
 - 1 mM N (Deficiency) vs 5 mM N (Control)
- **Measurements After Harvesting:**
 - Shoot Fresh Weight (g), Shoot Dry Weight (g) (SW)
 - Shoot N concentration (using DUMAS method)
 - Nit_{UI} (SW(SW/N content))
 - Nit_{UE} (SW/N content)

- **Association Mapping:**
 - Full set of densely SNP markers: 250,000 SNPs
 - Based on Linkage Disequilibrium
 - Therefore, detection of significant SNPs within 20kb will give us a QTL region which might be associated with NUE.

N deficiency

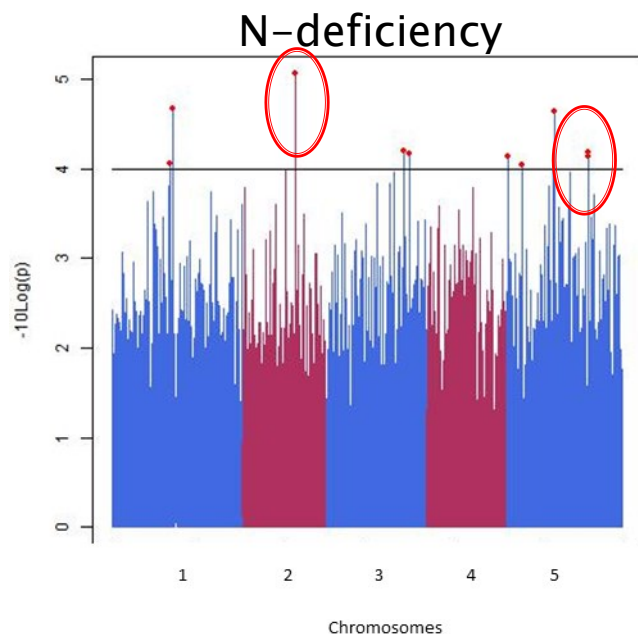
Control



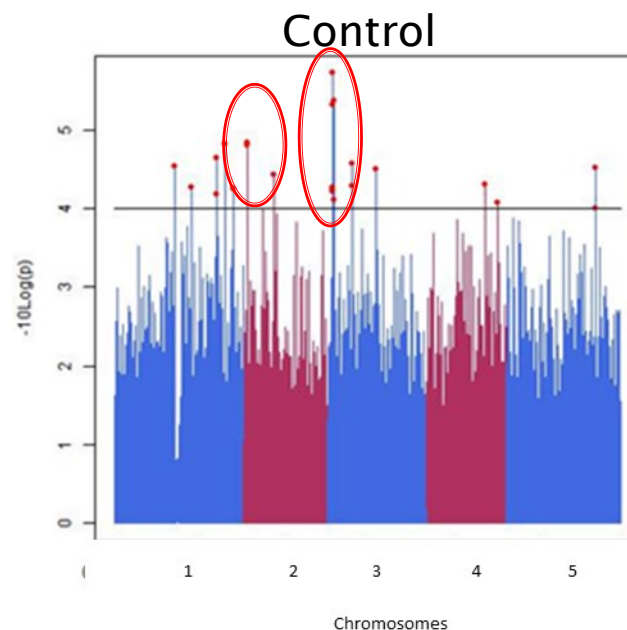
Association Mapping



GWA Mapping Nitrogen Use Efficiency

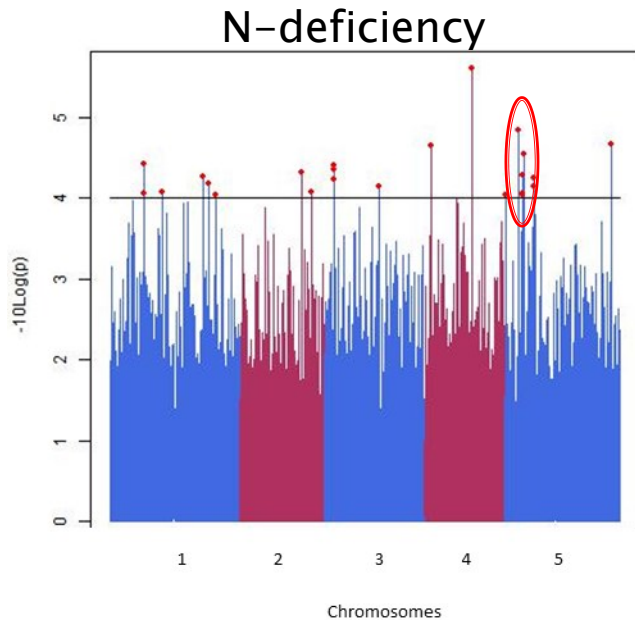


AT2G28800 Albino 3 (Alb3)
AT5G47120 Bax inhibitor 1 (BI-1)
AT5G47110 chlorophyll a/b
binding protein gene (LIL3:2)

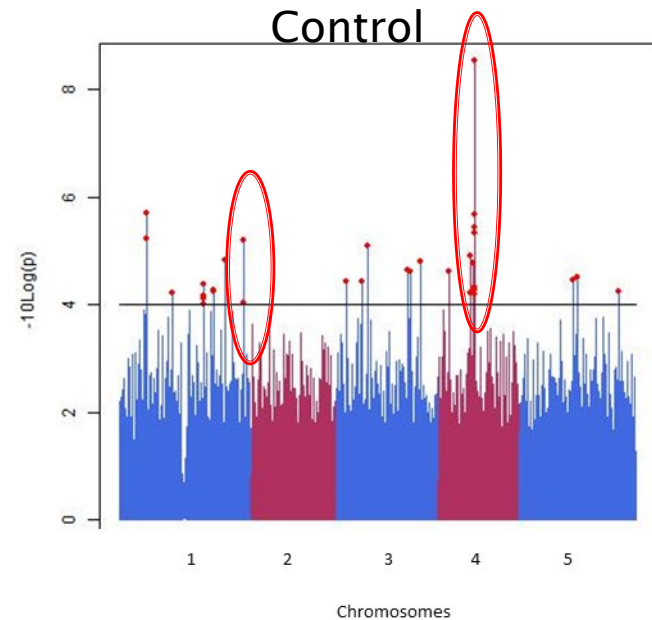


AT2G02590 Unknown
AT3G05090 Lateral Root Stimulator 1
(LRS1)

GWA Mapping Dry Weight



AT5G13550 Sulfate transporter
(SULTR4;1)

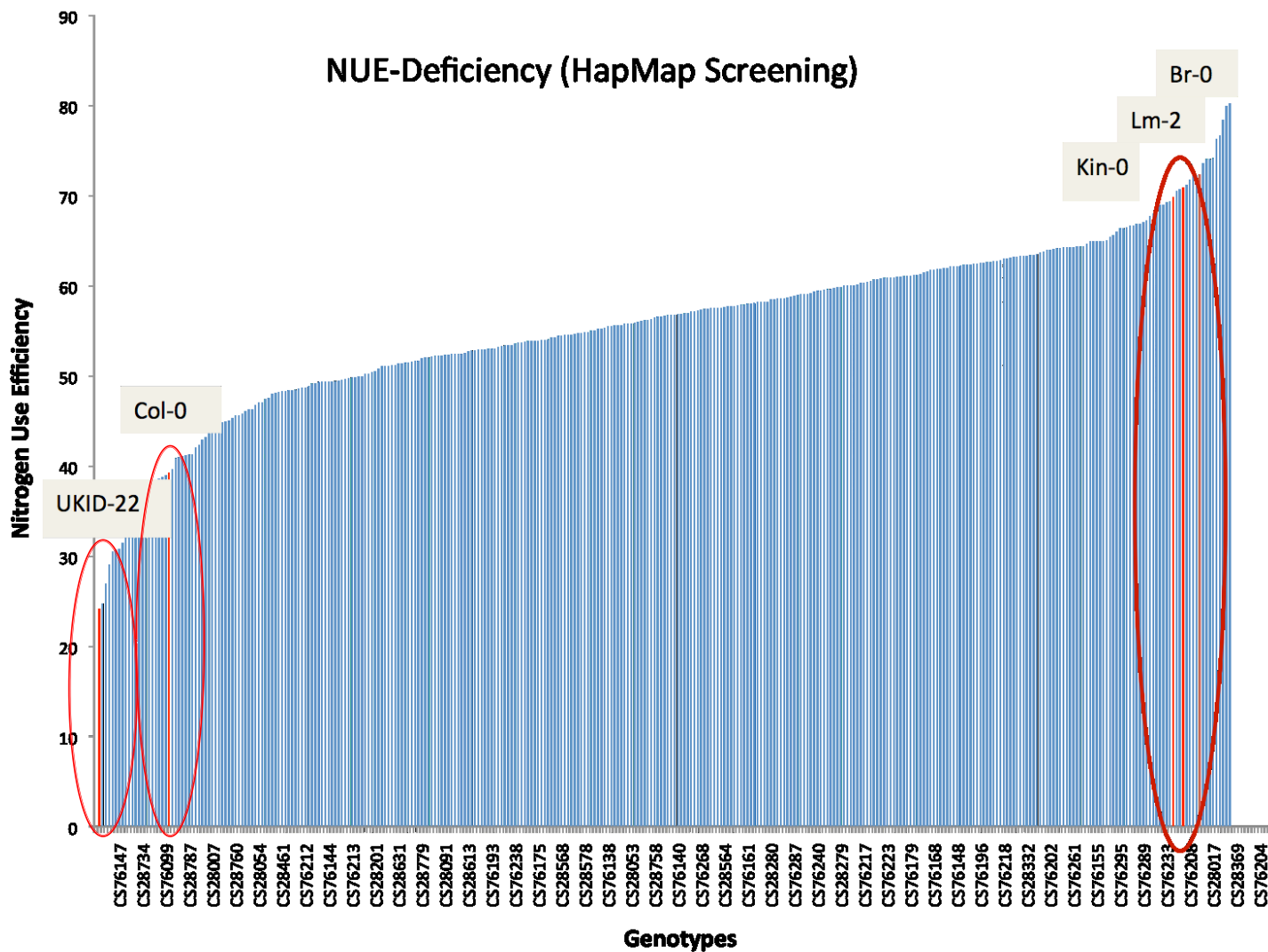


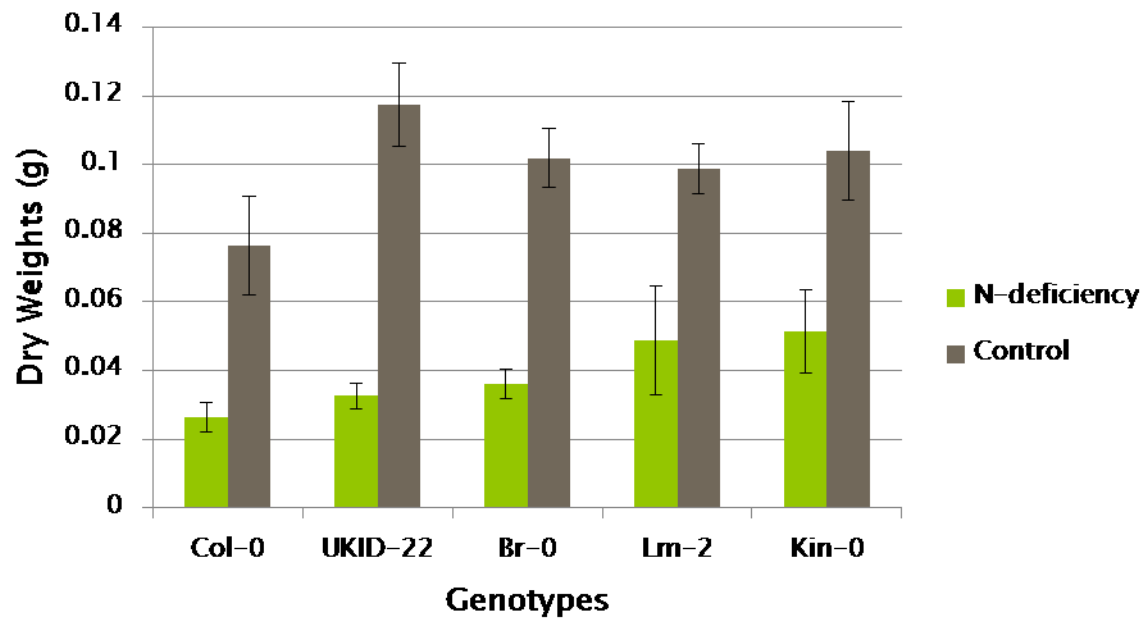
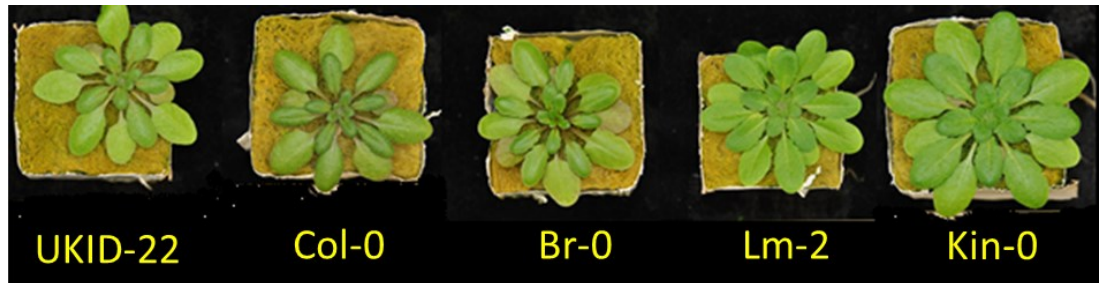
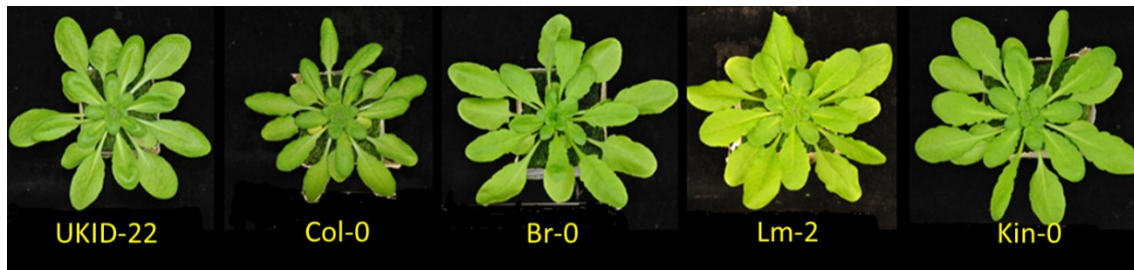
AT1G75960 AMP-dependent
synthetase and ligase family protein
AT4G14400 Accelerated cell death 6
(ACD6)

Growing Extreme Genotypes and Mutants



Ranking accessions





Summary *Arabidopsis*

Br-0 and Col-0 extreme genotypes

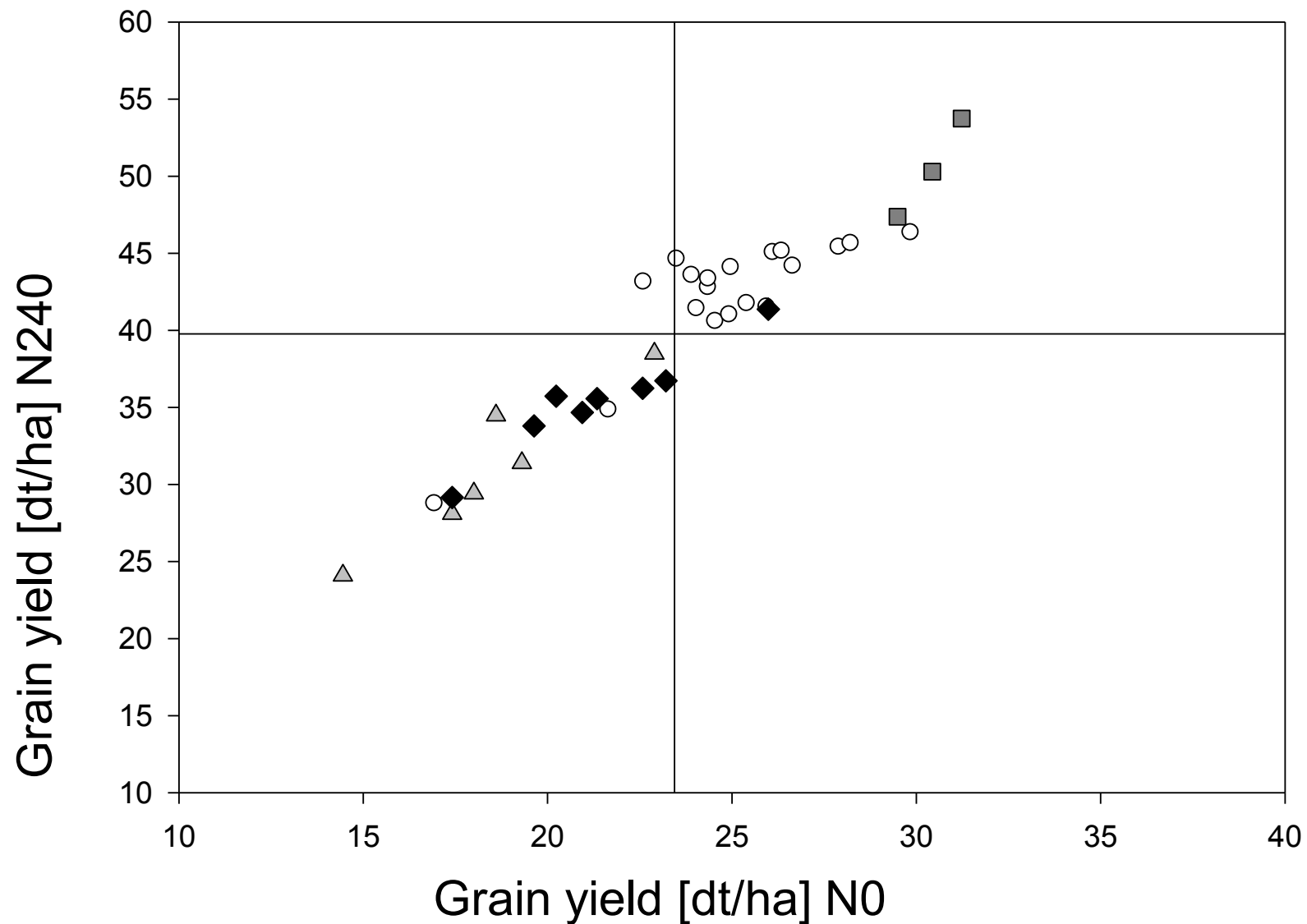
AT2G28800 Albino 3 (Alb3)
AT5G47120 Bax inhibitor 1 (BI-1)
AT5G47110 chlorophyll a/b binding protein gene (LIL3:2)
AT2G02590 Unknown
AT3G05090 Lateral Root Stimulator 1 (LRS1)
AT1G22170 Phosphoglycerate mutase family protein
AT2G14835 Ring/U box superfamily protein
AT5G13380 Auxin responsive GH3 family protein
AT4G14400 Accelerated cell death 6 (ACD6)
AT5G13550 Sulfate transporter (SULTR4;1)
AT1G75960 AMP-dependent synthetase and ligase family protein
AT1G52070 Mannose-binding lectine super family protein
AT3G46540 ENTH/VHS family protein
AT4G26990 Unknown
AT4G27010 Embryo defective 2788

15 candidates



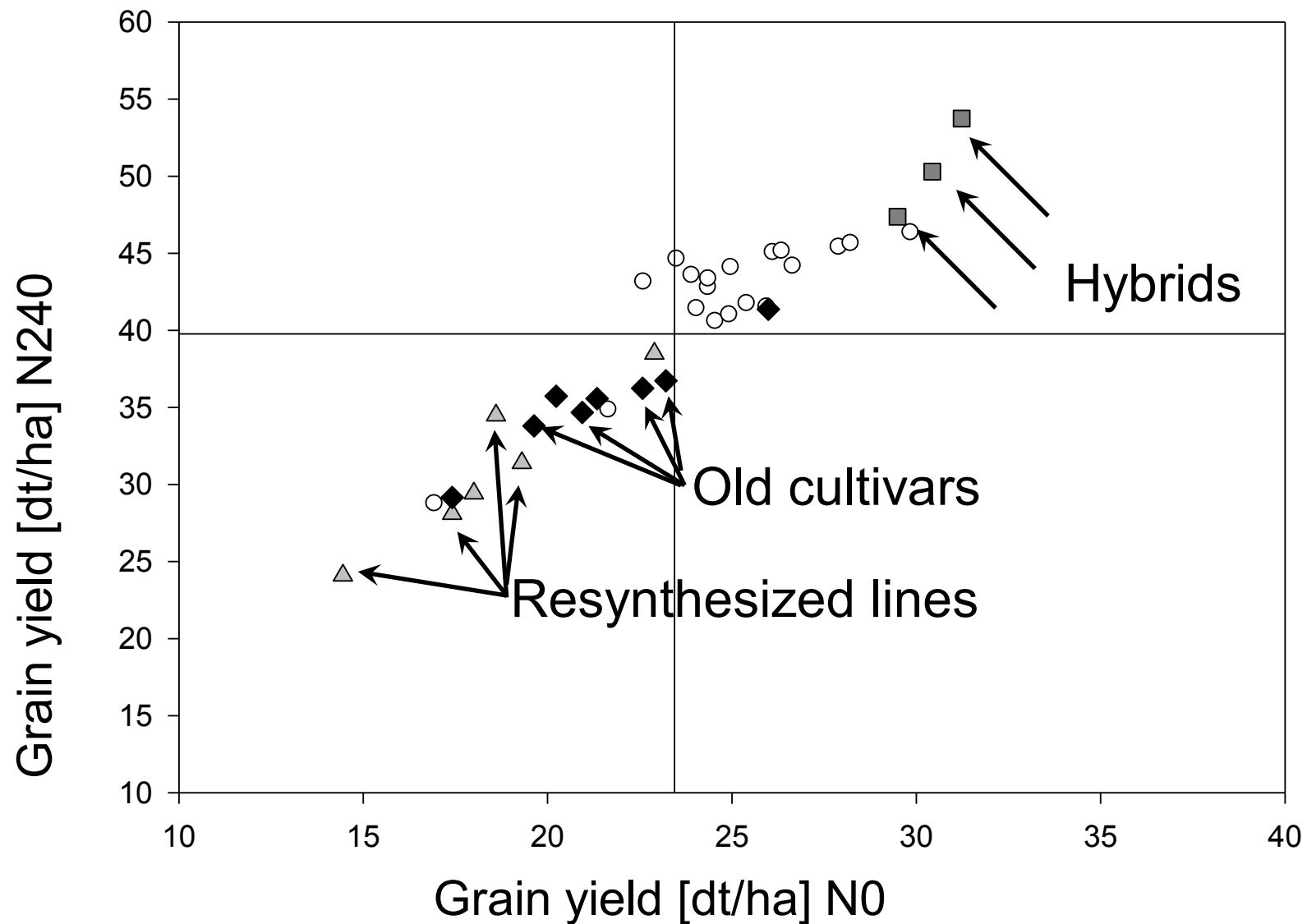
WP 3.3 Oilseed rape

Sebastian Miersch, Frank Breuer,
Andreas Gertz, Heiko Becker



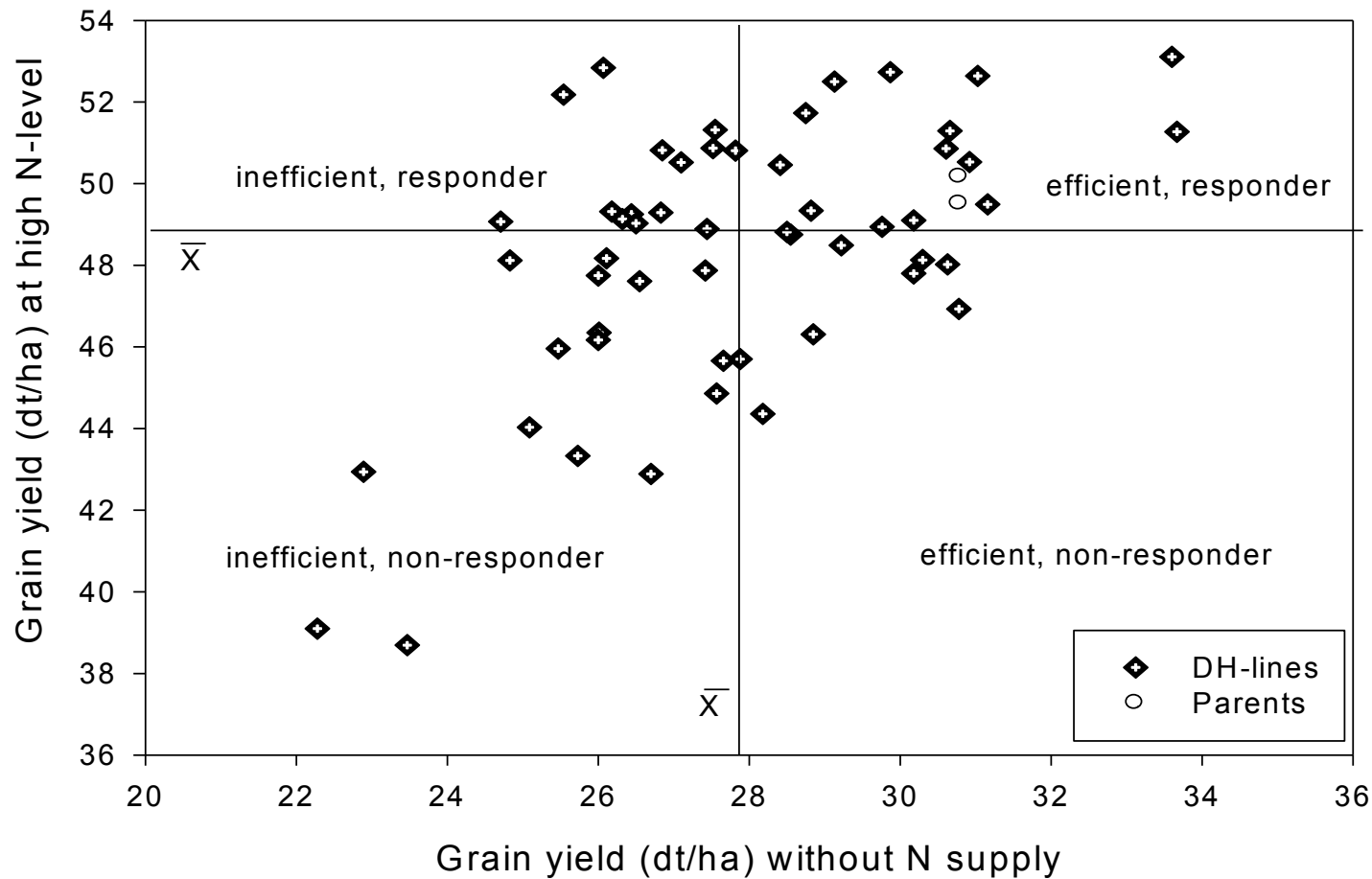
36 genotypes (resynthesized lines, old and new cultivars),
7 environments

KESSEL et al. 2012



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7 environments

KESSEL et al. 2012



54 DH lines (Apex x Mohican), 7 environments

NYIKAKO et al. 2003

Variance components for grain yield of oilseed rape grown at two N levels

Source of variation	Broad material (n=36)	DH Apex x Mohican (n=56)
Genotype	28.44	5.02
Genotype x location	6.82	2.61
Genotyp x N level	3.85	2.01

- In unselected material there is a considerable variation in N efficiency
- Large potential to select for N efficient cultivars

Are Semi-dwarf Hybrids a way to increase N efficiency?



Dwarf (ms mother)



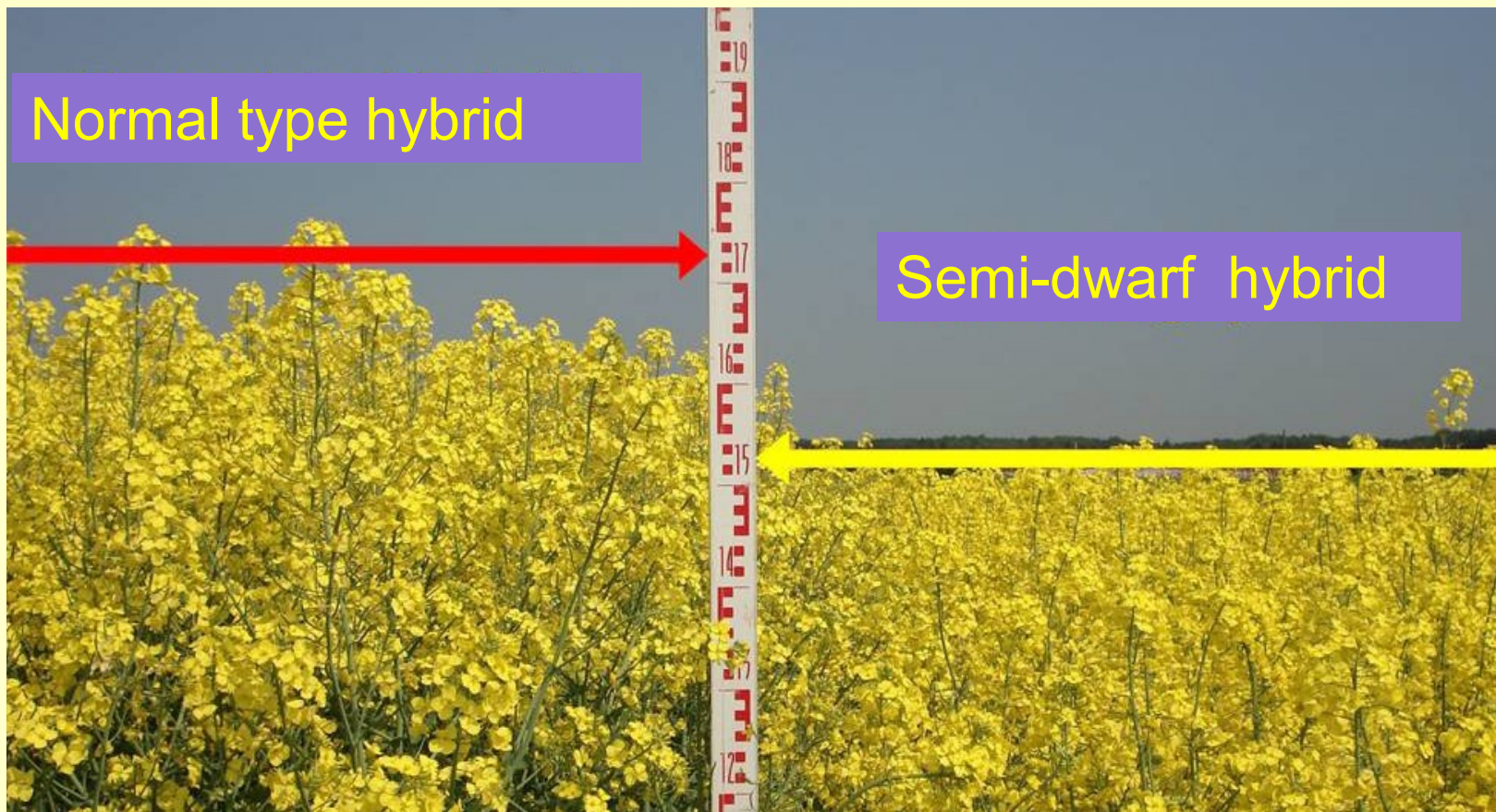
Semi-dwarf Hybrid



Normal type (pollinator)

Normal type hybrid

Semi-dwarf hybrid



Comparison of Semi-dwarf and normal hybrids

- A dwarf line was crossed with a normal line
- F1 derived DH-lines (242 DH-lines) segregate into **dwarf** and **normal types**
- all DH Lines were crossed to a normal male sterile tester to produce **normal** and **semi-dwarf testcross hybrids**
- development of plant material by KWS Saat AG



Field trials 2011/12 and 2012/13

- **108 testcross hybrids (54 semi dwarf and 54 normal types)**
were sown in replicated field trials in Einbeck and Göttingen
- **Selection of genotypes based on differences in N uptake from 2010/11**
- **cultivated under low nitrogen supply (no fertilization) and optimal nitrogen supply (160 kg N/ha)**
- **two harvest dates:**
 - end of flowering**
 - maturity – 2011/12 hail in Einbeck – no grain harvest only straw**



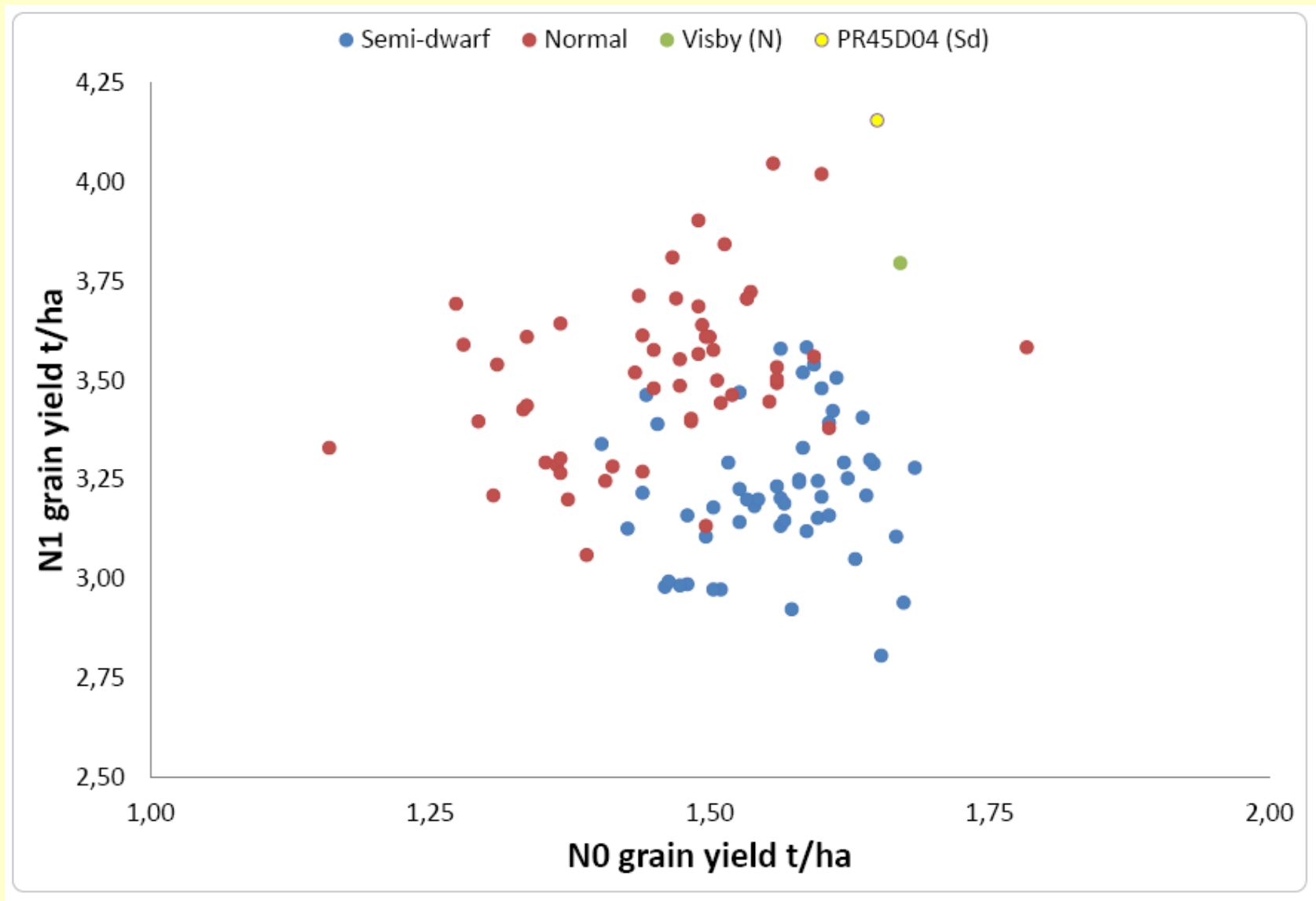
Linkage map

- A total of 881 (432 SNP, 109 SSR and 340 AFLP) polymorphic marker
- The final linkage map includes 438 markers (390 SNP, 49 SSR) distributed on 22 linkage groups (LG)
- 157 marker loci co-segregate
- 21 LGs could be assigned to the 19 chromosomes of the *Brassica napus* genome
- total map length of 1301 cM (Kosambi)

Results

Mean values of 54 semi-dwarf and 54 normal-type testcrosses (mean of 3 environments)

		Grain t/ha DM		Straw t/ha DM		HI	
N0	Semi-dwarf	1.56	**	2.87	**	0.35	**
	Normal	1.45		3.48		0.29	
N1	Semi-dwarf	3.22	**	3.94	**	0.45	
	Normal	3.52		4.36		0.45	



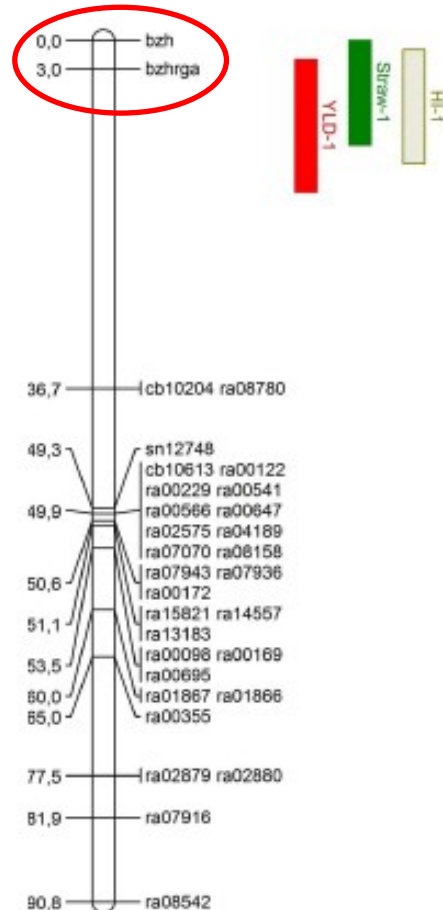
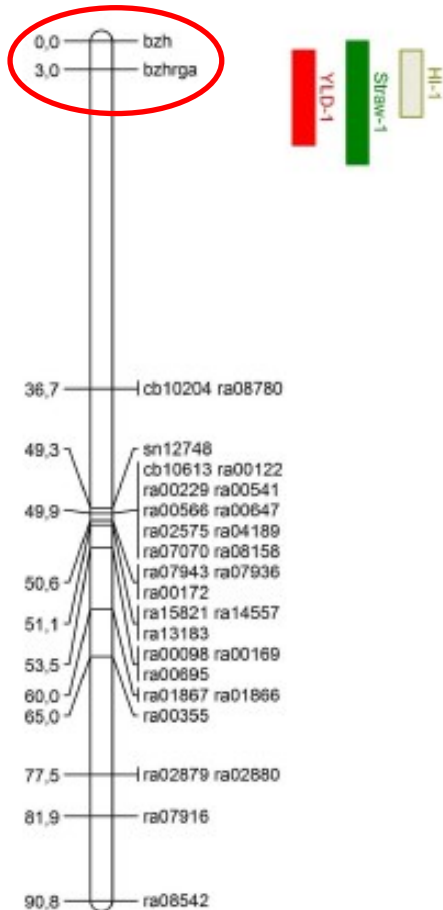
Yield of 54 semi-dwarf and 54 normal-type testcrosses (3 environments)

N0

N1

N06

N06



Major QTL for grain yield, straw yield and HI in the region of the dwarf gene

Conclusion and Outlook

- Nit-UE is a complex trait with considerable genetic variation
- Plant architecture (semi-dwarf) is of interest
- Role of root system is still largely unexplored
- Association mapping and QTL mapping are useful tools
- Combined analysis of all data from this sub-project is a challenge for the next few months

Root system size measured by electrical capacitance (Chloupek 1977)



Photo: Julia
RUDLOFF

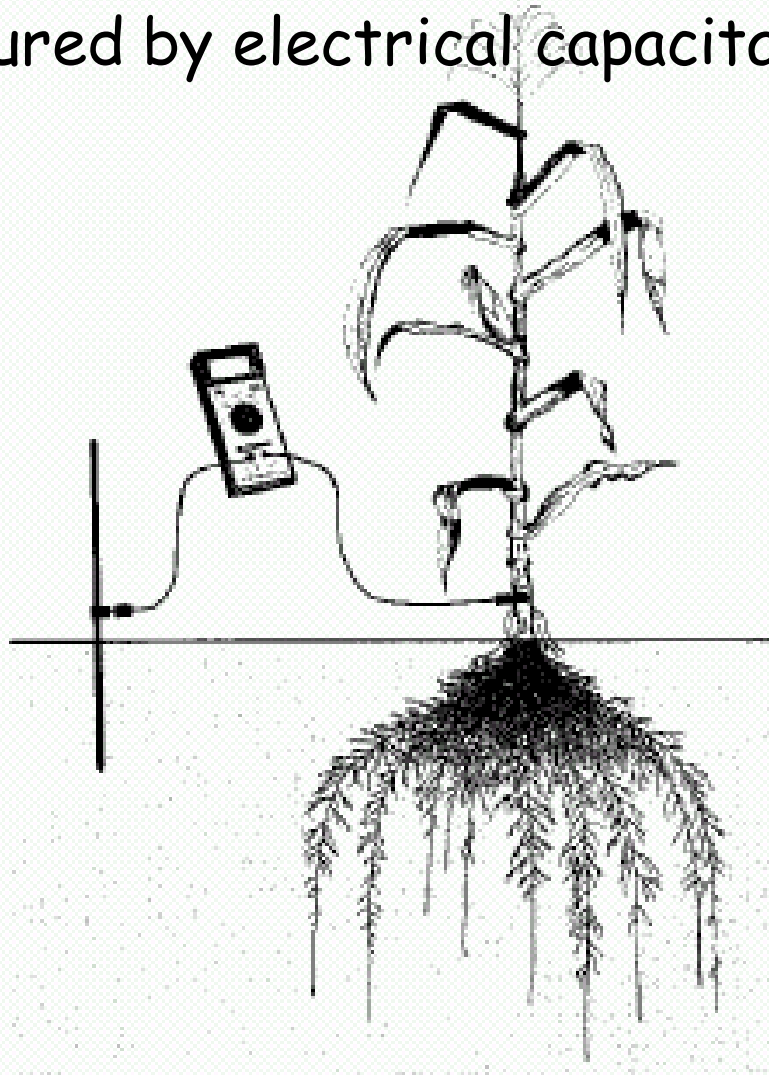


Fig. 1. Estimation of root system size with a BK Precision 810A capacitance meter. The negative electrode is attached to the maize stem 6 cm above ground level and the positive electrode is attached to a copper ground rod.

VAN BEEM et al.
1998

Thank you for your attention

