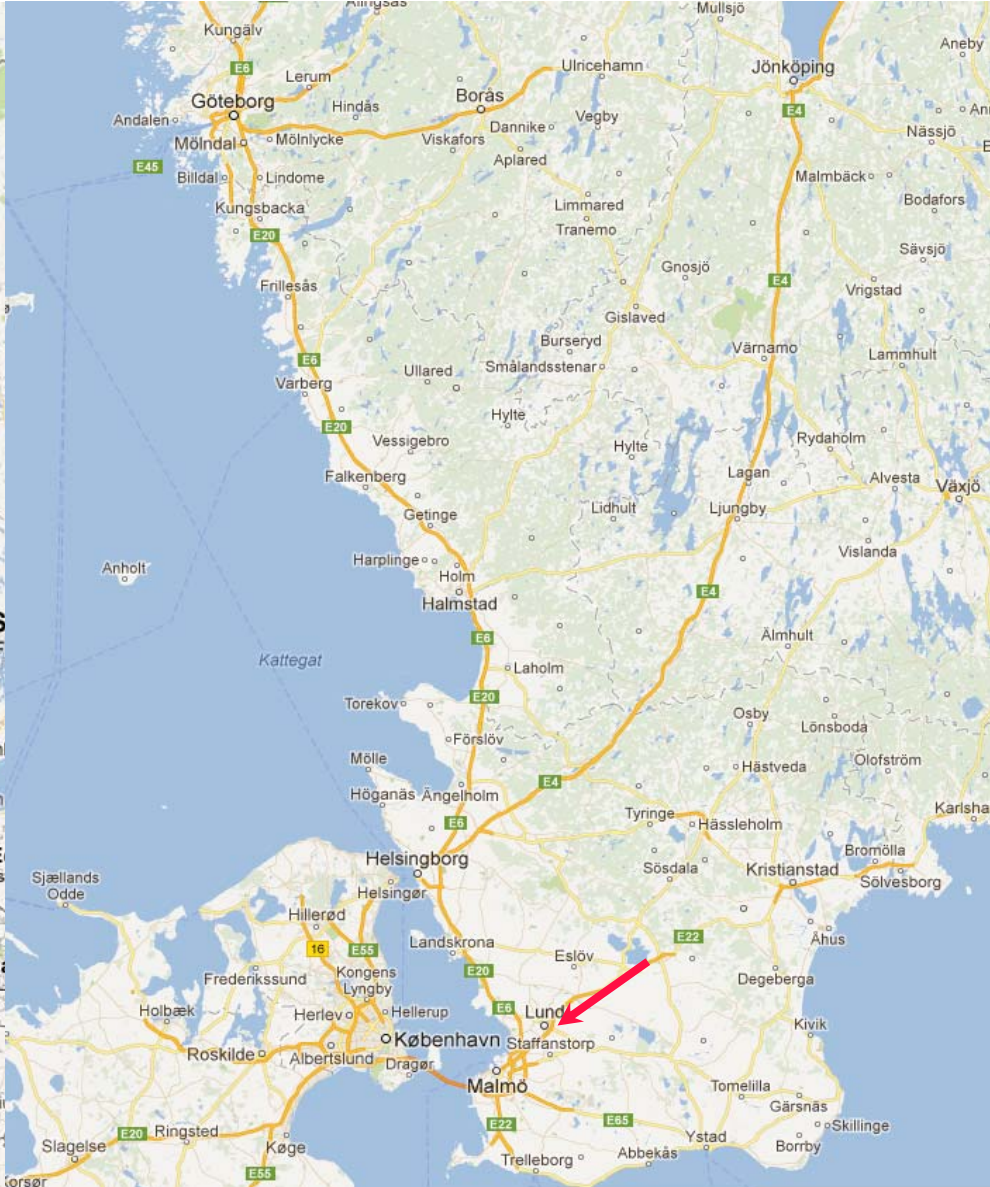


Molecular breeding for enhanced food quality in oat

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Mutagenesis has become popular during recent years

Species ^a	Year ^b	Mutagen ^c	Mutation rate ^d	Screening method ^e	Source ^f
<i>Arabidopsis</i>	2000	EMS	1/153 Kb	dHPLC, Li-Cor	McCallum et al. [24], Colbert et al. [25]
Rice	2001	DEB, GR, FN	1/40 Kb (deletion)	Phenotypic (stress)	Leung et al. [16]
<i>Lotus japonicus</i>	2003	EMS	1/502 Kb	Li-Cor, CE	Perry et al. [26, 27]
<i>Arabidopsis</i>	2003	EMS	1/208 Kb	Li-Cor	Till et al. [28]
Barley	2004	EMS	1/1 Mb	dHPLC	Caldwell et al. [23]
Maize	2004	EMS	1/485 Kb	Li-Cor	Till et al. [19]
Durum wheat	2005	EMS	1/40 Kb	Li-Cor	Slade et al. [29]
Bread wheat	2005	EMS	1/24 Kb	Li-Cor	Slade et al. [29]
Rice	2005	EMS	1/1 Mb	Li-Cor	Wu et al. [17]
Rice	2007	EMS	1/294 Kb	Li-Cor	Till et al. [18]
Rice	2007	Az-MNU	1/265 Kb	Li-Cor	Till et al. [18]
Pea	2007	EMS	1/669 Kb	Li-Cor	Triques et al. [30]
Soybean	2008	EMS	1/140 Kb	Li-Cor	Cooper et al. [31]
Soybean	2008	NMU	1/140 Kb	Li-Cor	Cooper et al. [31]
Rice	2008	MNU	1/135 Kb	CE	Suzuki et al. [32]
Barley	2008	Az	1/374 Kb	Li-Cor	Talamè et al. [33]
Rapeseed	2008	EMS	1/41 Kb	Li-Cor	Wang et al. [34]
Sorghum	2008	EMS	1/526 Kb	Li-Cor	Xin et al. [35]
Bread wheat	2008	EMS	1/23 Kb	AGE	Dong et al. [36]
Tomato	2009	EMS	1/735 Kb	CE, HRM	Gady et al. [37]
Barley	2009	EMS	1/500 Kb	Li-Cor	Gottwald et al. [38]
Cabbage	2009	EMS	1/447 Kb	Li-Cor	Himelblau [39]
Medicago	2009	EMS	1/424 Kb	CE	Le Signor et al. [40]
Medicago	2009	EMS	1/485 Kb	Li-Cor	Le Signor et al. [40]
<i>Arabidopsis</i>	2009	EMS	1/51 Kb	Li-Cor	Martín et al. [41]
Bread wheat	2009	EMS	1/40 Kb	PAGE	Uauy et al. [42]
Bread wheat	2009	EMS	1/41 Kb	Li-Cor	Uauy et al. [42]
<i>Arabidopsis</i>	2010	EMS	1/415 Kb	HRM	Bush and Krysan [43]
Melon	2010	EMS	1/573 Kb	Li-Cor	Dahmani-Mardas et al. [44]
Pea	2008	EMS	1/200 Kb	Li-Cor	Dalmais et al. [45]
Oat	2010	EMS	1/30 Kb	MALDI-TOF	Chawade et al. [22]
Tomato	2010	EMS	1/322 Kb	Li-Cor	Minoia et al. [46]
Bread wheat	2010	EMS	NA	Li-Cor	Sestili et al. [47]
<i>Brassica rapa</i>	2010	EMS	1/44 Kb	CE	Stephenson et al. [48]
Peanut	2011	EMS	1/931 Kb	Li-Cor	Knoll et al. [49]
Peanut	2011	DES	None detected	Li-Cor	Knoll et al. [49]
Sunflower	2011	EMS	1/475 Kb	Li-Cor	Sabetta et al. [50]

RESEARCH ARTICLE

Open Access

Development and characterization of an oat TILLING-population and identification of mutations in lignin and β -glucan biosynthesis genes

Aakash Chawade^{1,2}, Per Sikora², Marcus Bräutigam^{1,3}, Mikael Larsson⁴, Vivekanand Vivekanand², Montedar Ali Nakash³, Tingsu Chen^{1,5} and Olof Olsson^{*2,3}

Abstract

Background: Oat, *Avena sativa* is the sixth most important cereal in the world. Presently oat is mostly used as feed for animals. However, oat also has special properties that make it beneficial for human consumption and has seen a growing importance as a food crop in recent decades. Increased demand for novel oat products has also put pressure on oat breeders to produce new oat varieties with specific properties such as increased or improved β -glucan-, antioxidant- and omega-3 fatty acid levels, as well as modified starch and protein content. To facilitate this development we have produced a TILLING (Targeting Induced Local Lesions IN Genomes) population of the spring oat cultivar SW Belinda.

Mutation breeding; advantages

- ★ Increase variation in elite market varieties
- Creates variation without a loss of domesticated genes
- Very powerful in combination with high precision selection methods (molecular or biochemical)
- Possible to use sophisticated screens since the number of required analysis goes down with increased variation

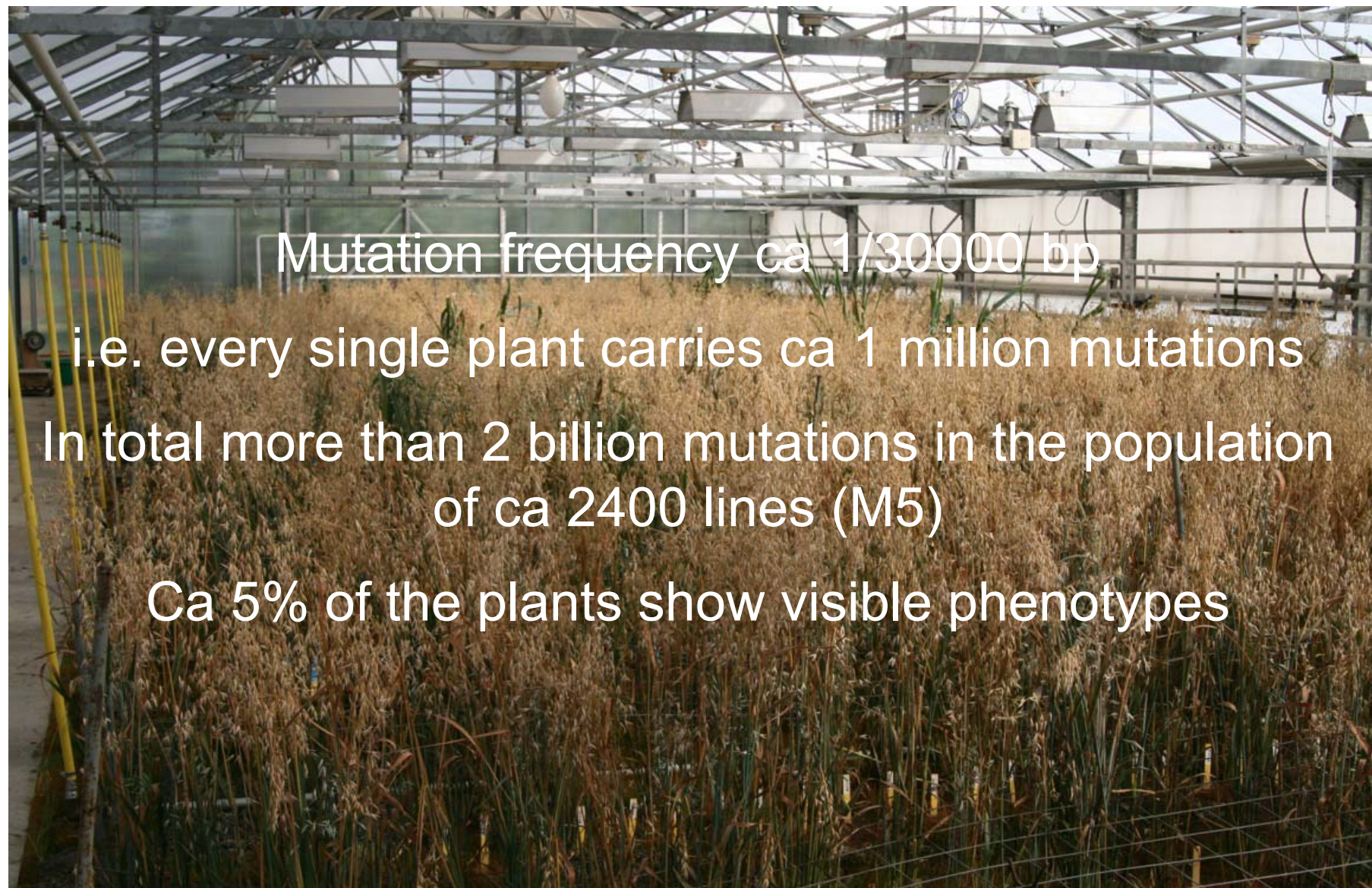
Oat mutagenised population

Mutation frequency ca $1/30000$ bp

i.e. every single plant carries ca 1 million mutations

In total more than 2 billion mutations in the population
of ca 2400 lines (M5)

Ca 5% of the plants show visible phenotypes

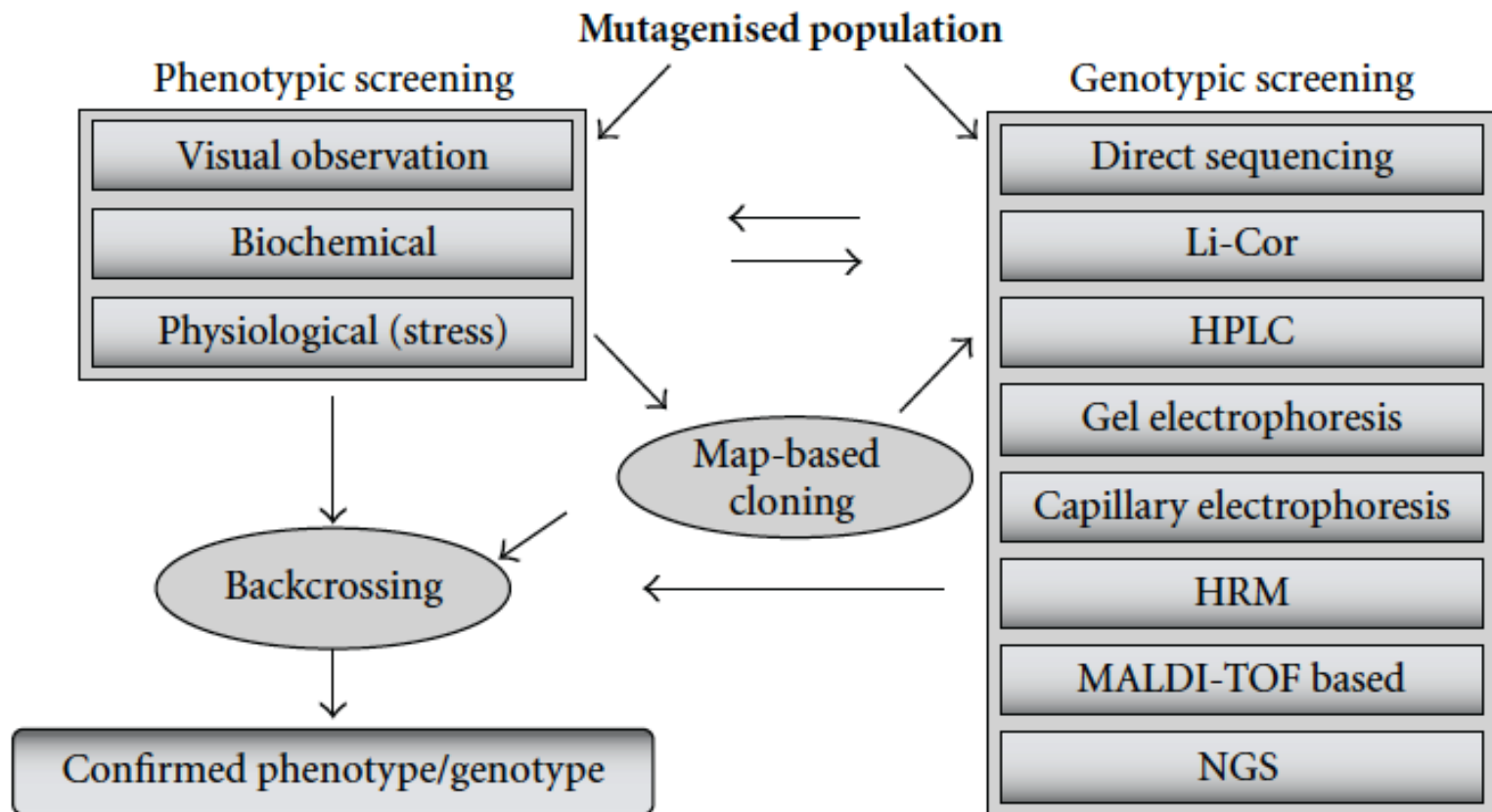


TILLING-population in the field

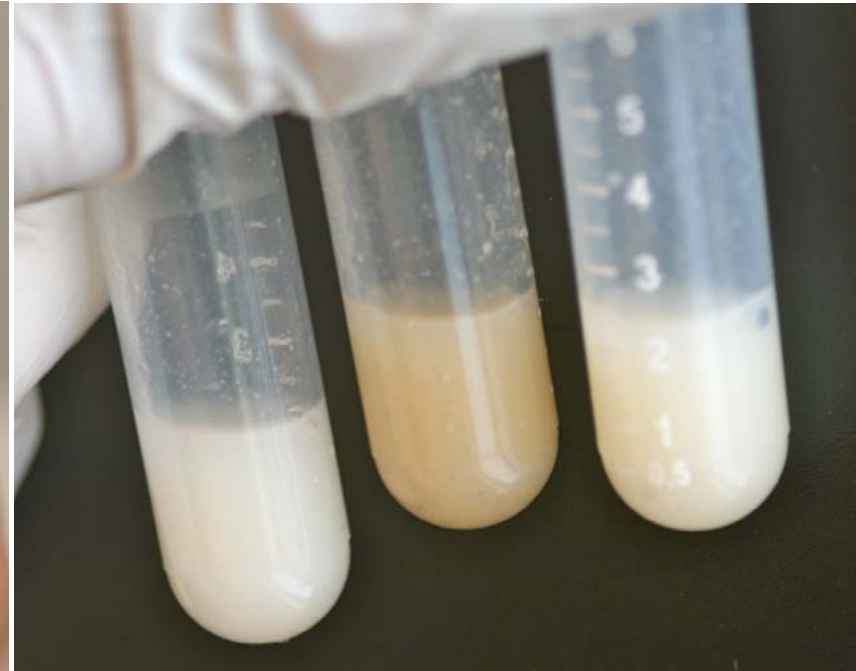
Most plant look normal



Screening possibilities



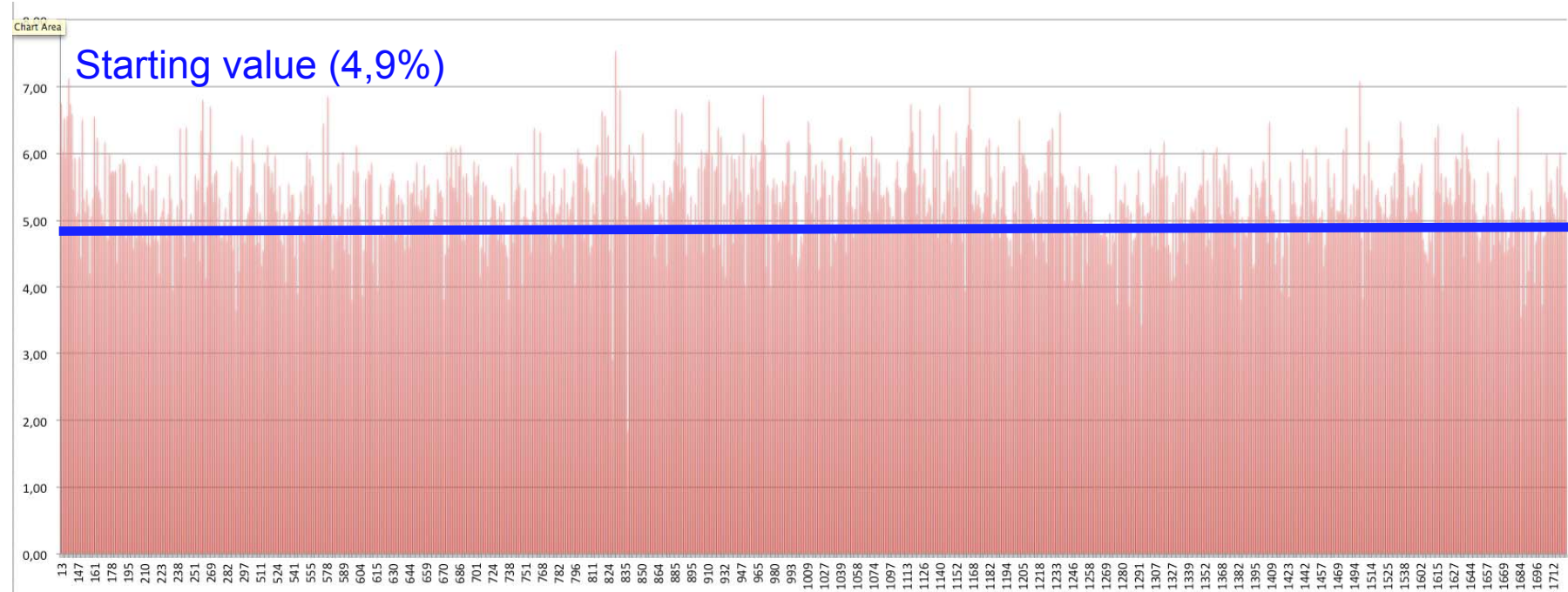
Development of a β -glucan miniassay



Powder from a few or single seeds was assayed by a modified Megazyme assay (industrial standard for β -glucan)

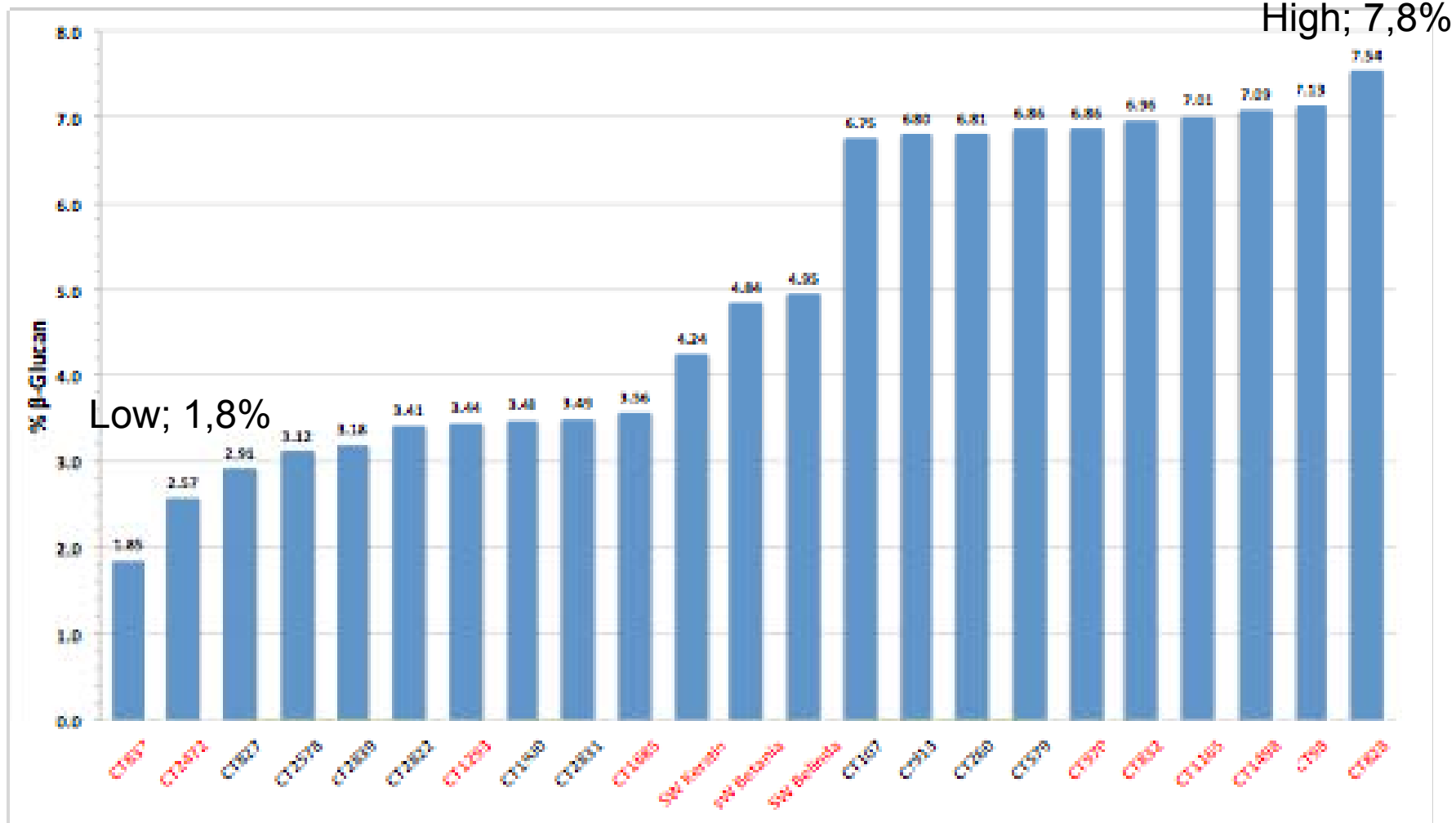
β -glucan levels in 1700 random lines

% β -glukan



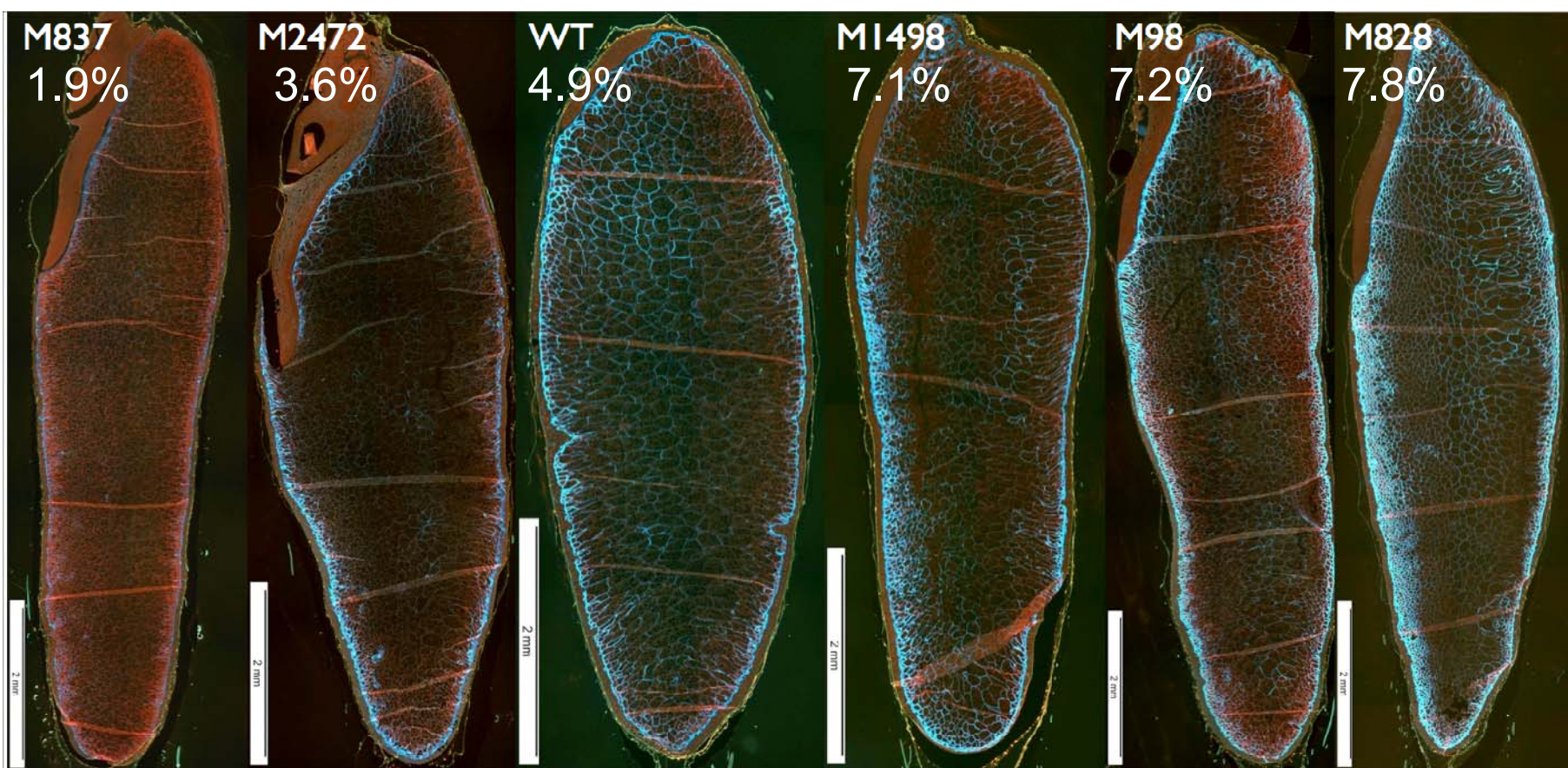
The variation is very high

Highest and lowest lines



Lines selected for further study in red

Localisation of β -glucans in oat seeds

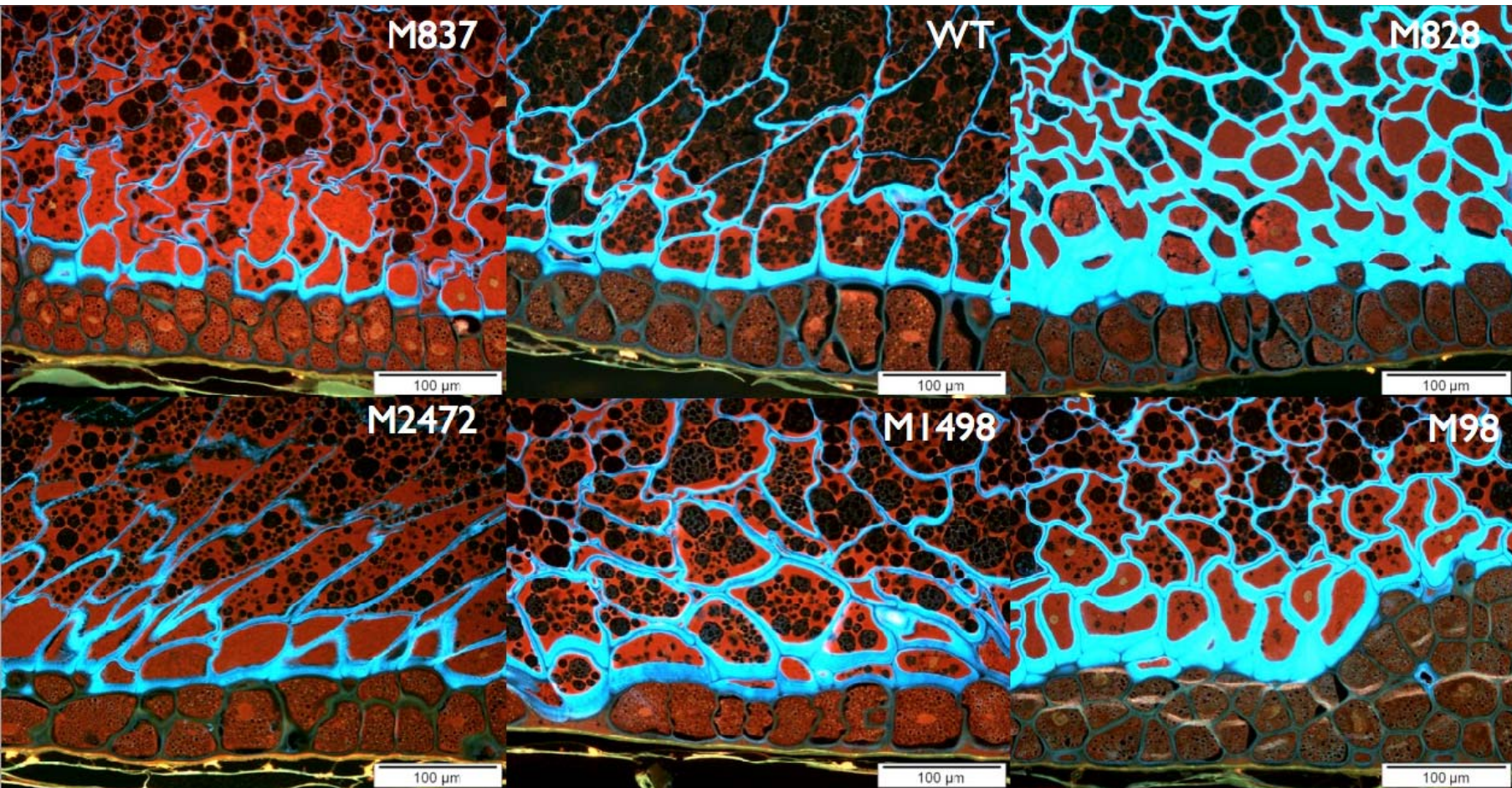


Low lines

Belinda

High lines

Zooming in on high and low lines



Longitudinal sections (partial)

For more details, see poster P29; Sikora P. et al

Continued project

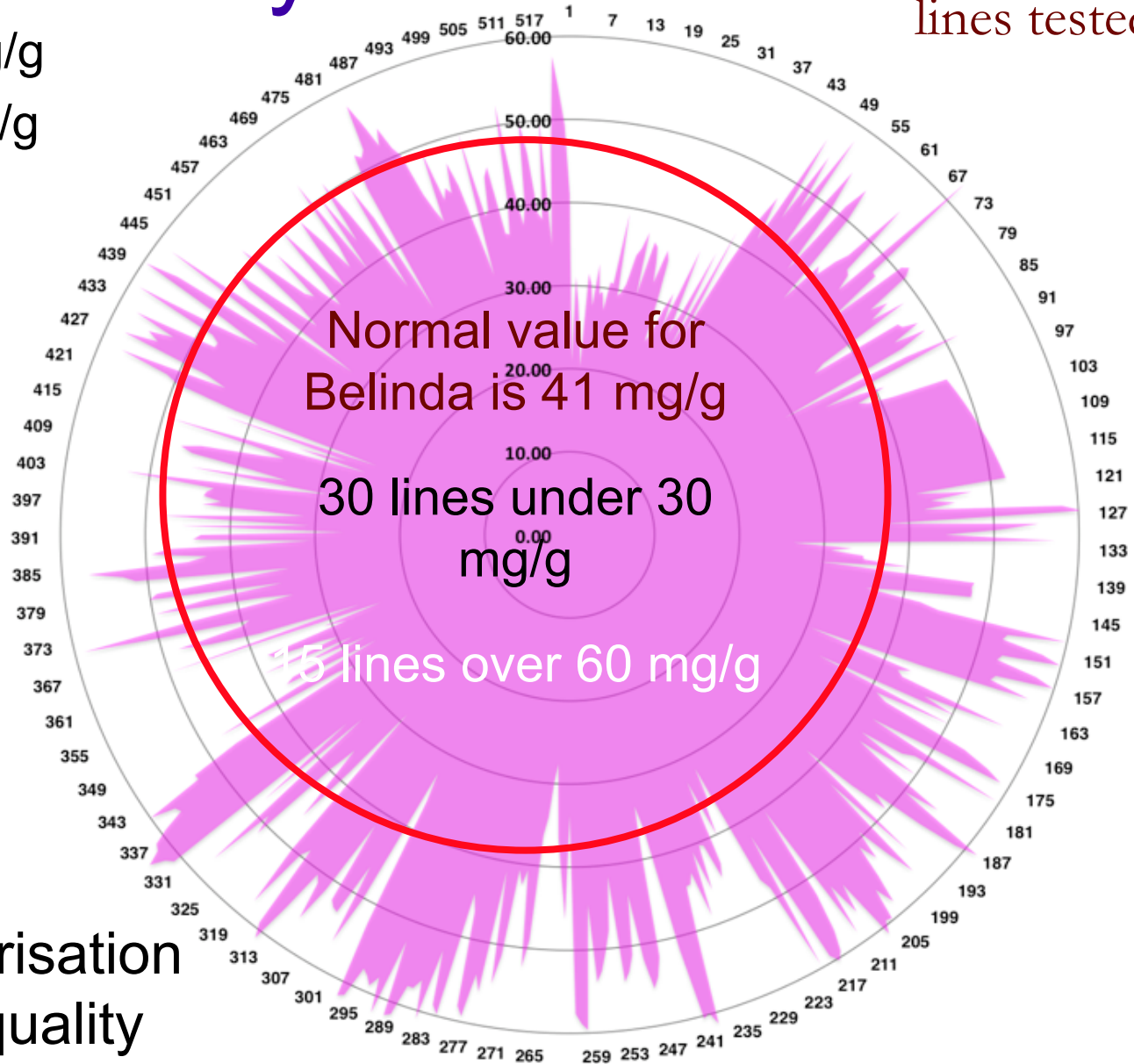
- Screening for oats with different or very different β -glucan quality
- Testing physiologic response (cholesterol lowering ability) of different β -glucan qualities in a mouse model system

Lignin assay

520 random
lines tested

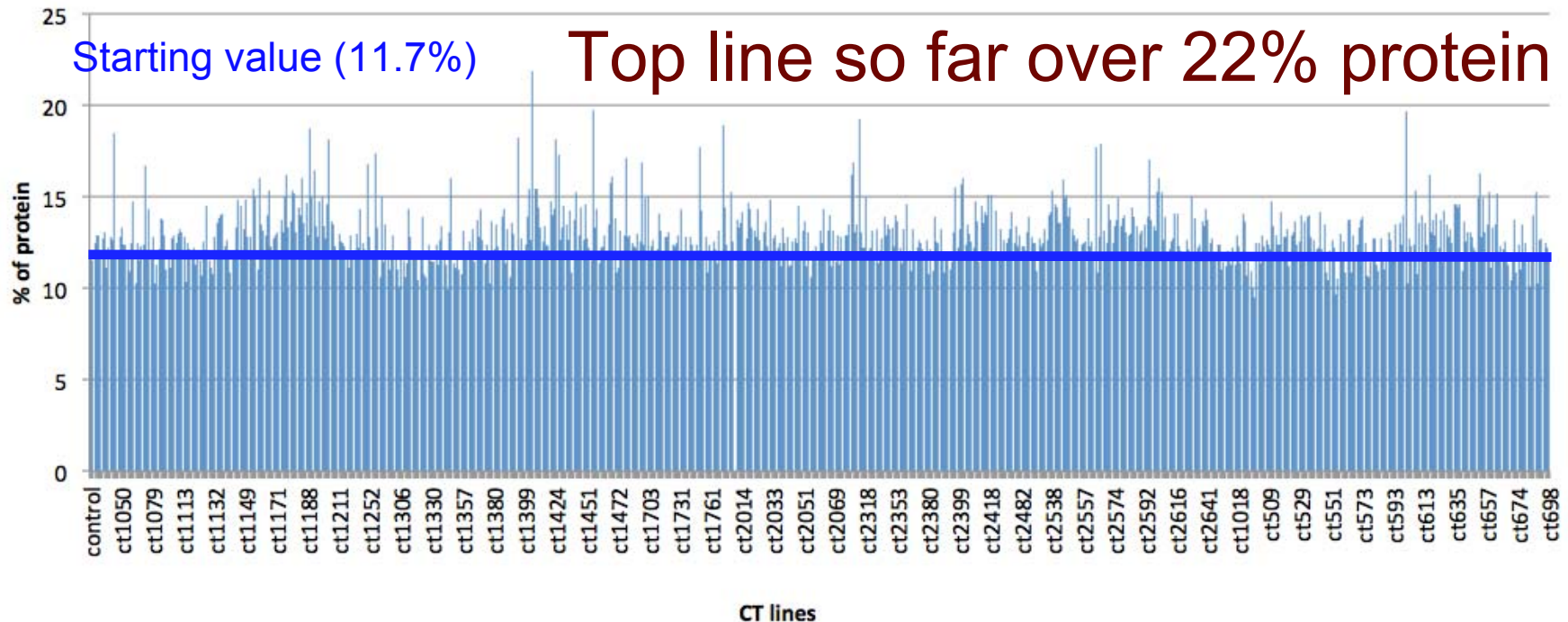
High; 63 mg/g

Low; 20 mg/g



Characterisation
of lignin quality

Total protein levels in 750 lines determined by an elemental analyzer (N)

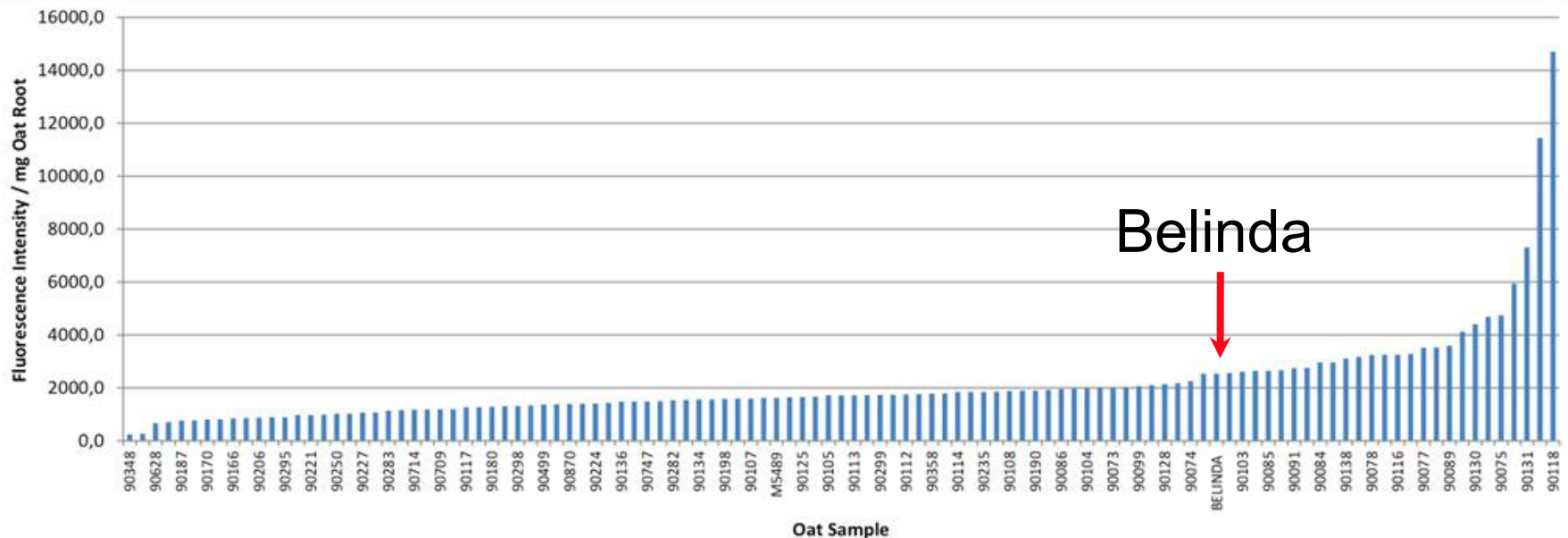


Next - study 2D gel pattern, aa composition, proportion of globular proteins, regulation, reological properties, etc

Sapponins

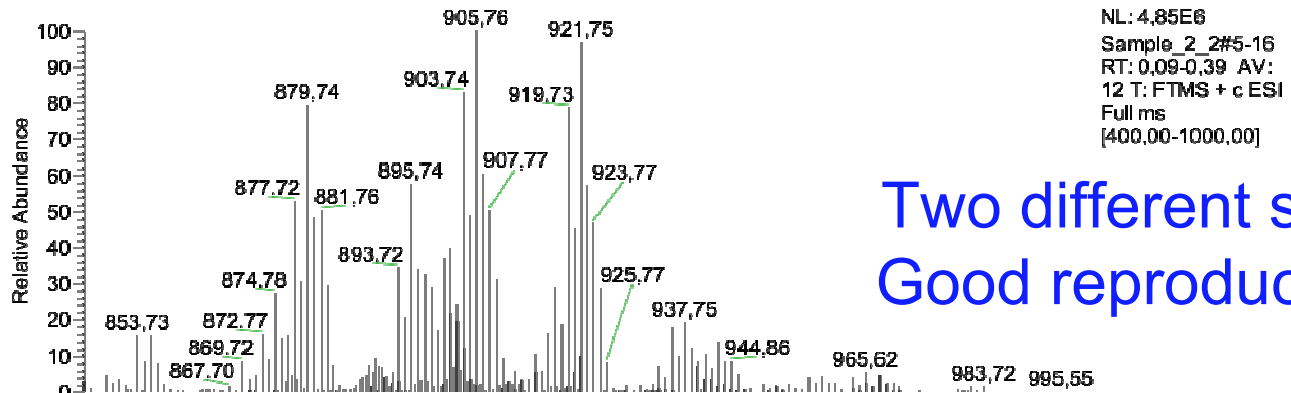
Fluorescence Intensity of Oat Roots samples.
Indirect measurement of Avenacin content.

111 samples including Belinda
 $\lambda_{\text{emission}}$ 460 nm ; $\lambda_{\text{excitation}}$ 390 nm



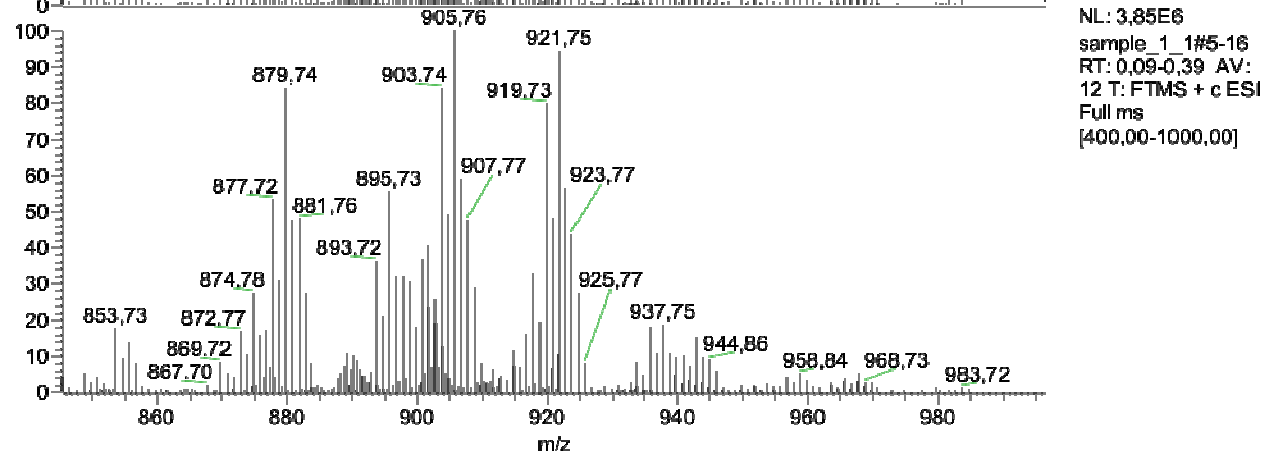
Orbitrap MS analysis of a non-polar extract of Belinda seeds

Sample 1



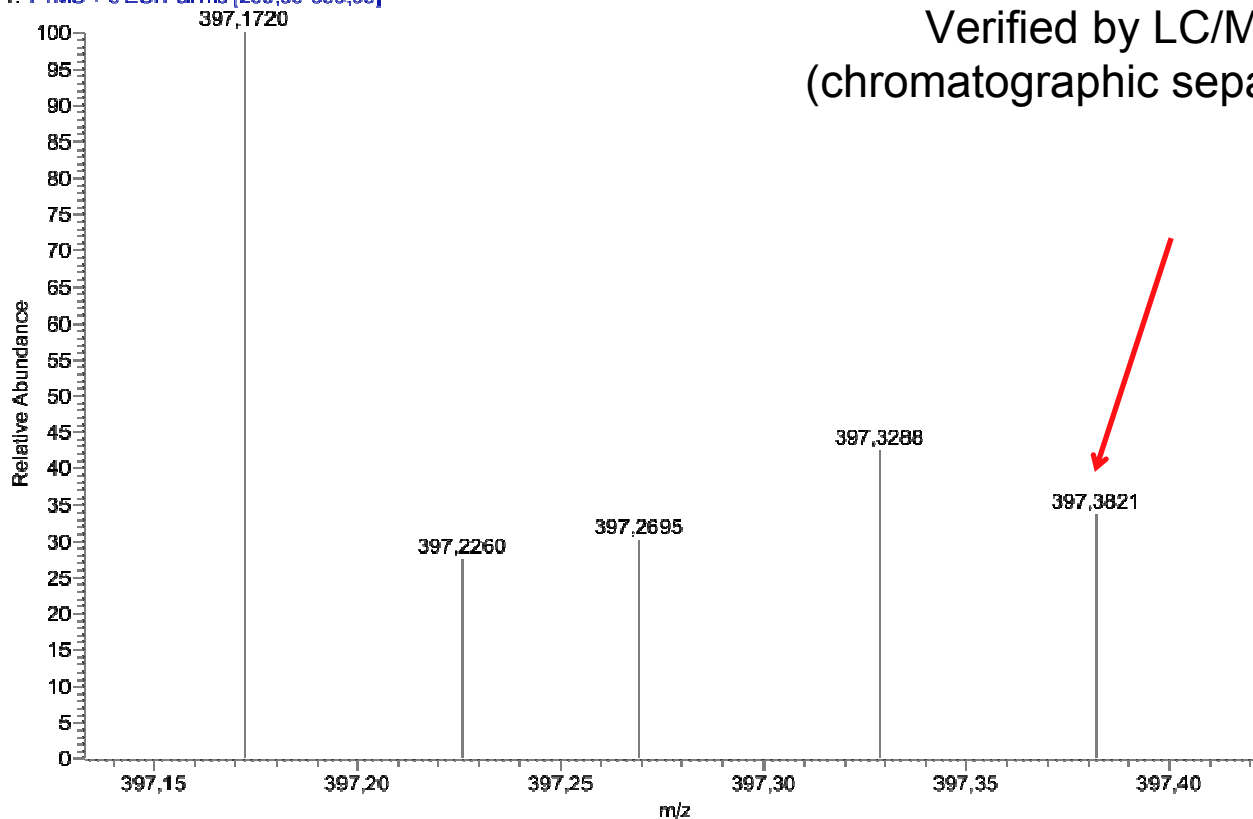
Two different seeds
Good reproducibility

Sample 2



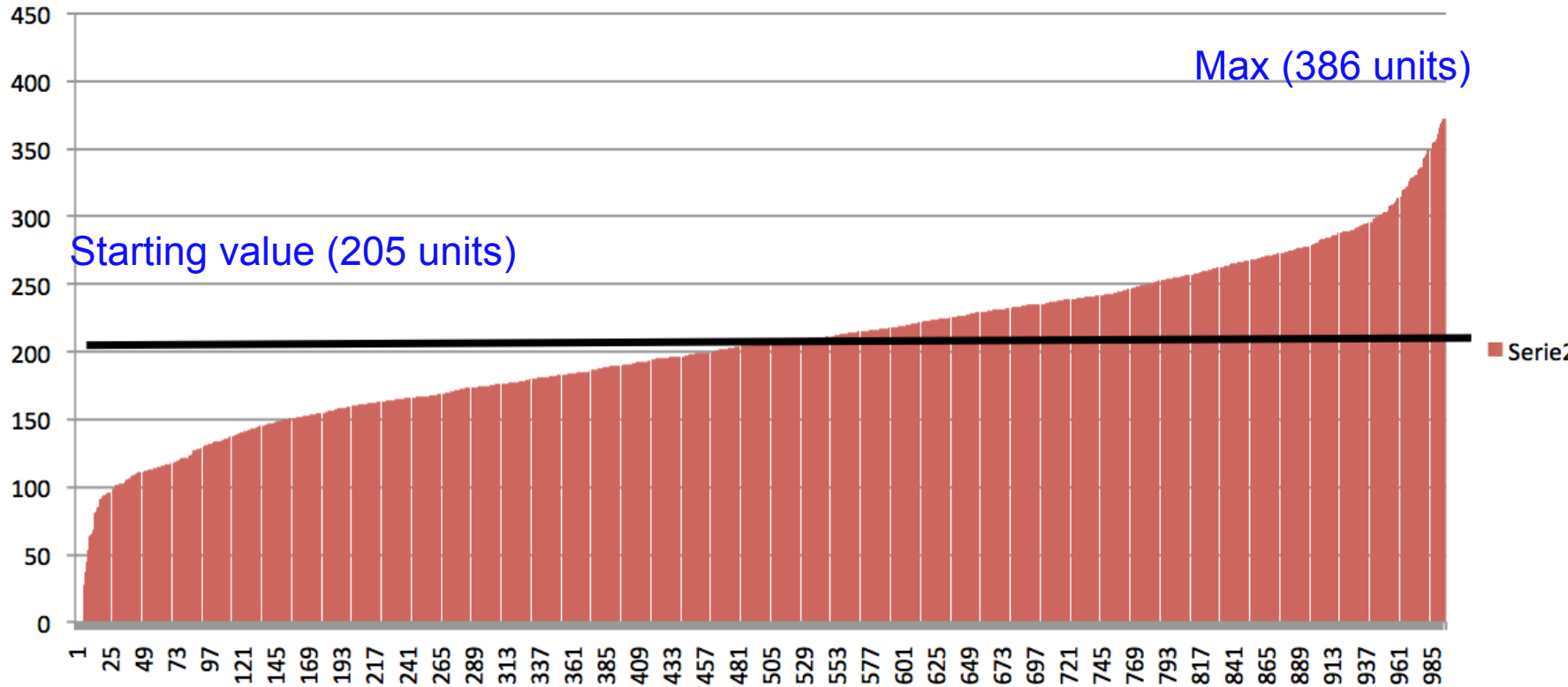
Identification of the β -sitosterol peak

Sample 1_L #6-14 RT: 0,12-0,34 AV: 9 NL: 1,04E4
T: FTMS + c ESI Full ms [200,00-600,00]



Fragment of sitosterol (loss of OH)
Verified by LC/MS
(chromatographic separation)!

Relative β -sitosterol levels in 1000 random lines

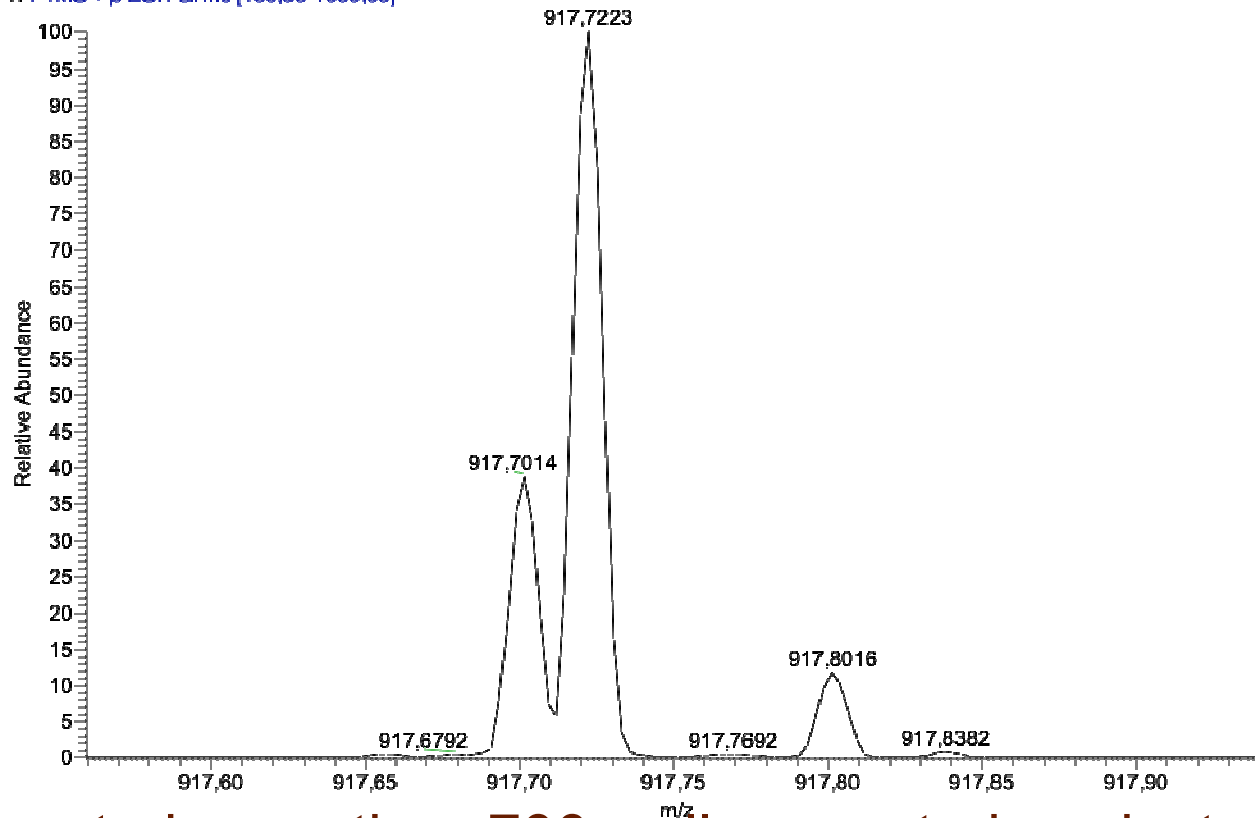


Min (28 units)

Range from less than half to double
normal levels. Variation is very high!

The high resolution of the orbitrap makes it possible to separate nearly all the peaks

Lip_sample_8_contium-mode #5-16 RT: 0,09-0,39 AV: 12 NL: 2,11E6
T: FTMS + p ESI Full ms [400,00-1000,00]



We converted more than 700 well separated peaks to numerical values. We then compiled spectra from all 1000 samples into one graph

Example of a dot-plot (70 peaks)

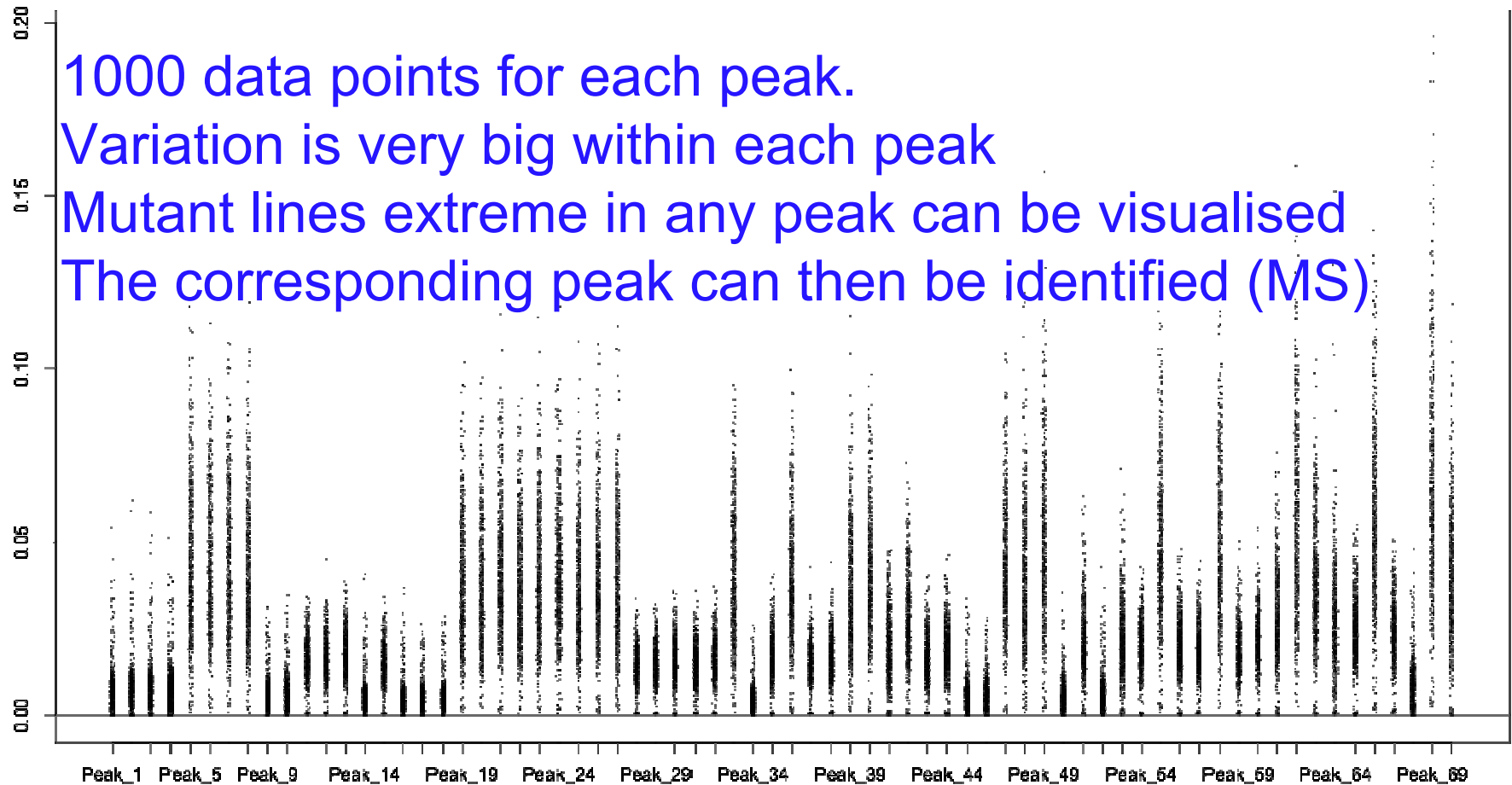
Lipid Profiles

1000 data points for each peak.

Variation is very big within each peak

Mutant lines extreme in any peak can be visualised

The corresponding peak can then be identified (MS)



Other projects

- Abiotic stress (cold tolerance)
 - ✿ See poster p30; Chawade A. et al
- Fusarium resistance
 - ✿ See poster p31; Olsson J. et al

Conclusion

- We have produced a mutagenised population of oat with a very high variation
- With the appropriate assay we most likely can identify lines weaker or stronger **in any character we are looking for!**

Phenotypic screening

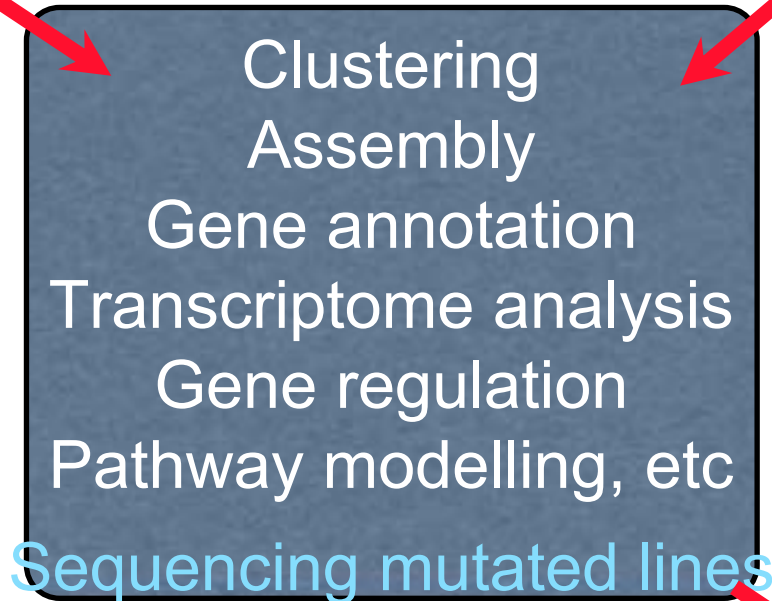
- Advantage
 - ✿ Gives trait directly.
 - ★ Variety development
 - ✿ Since both high and low lines are identified, a "model system" to study the mechanisms behind the trait is created
 - ★ Basic science
- Disadvantage
 - ✿ Specific SNPs correlated to the phenotype (trait) has to be identified before molecular marker assisted selection can take place

From phenotype to genotype

Raw mRNA
sequences
(ESTs)

micro-RNA
sequences

Presently we
have ca 181
million reads in
total ca
17,1 gbases

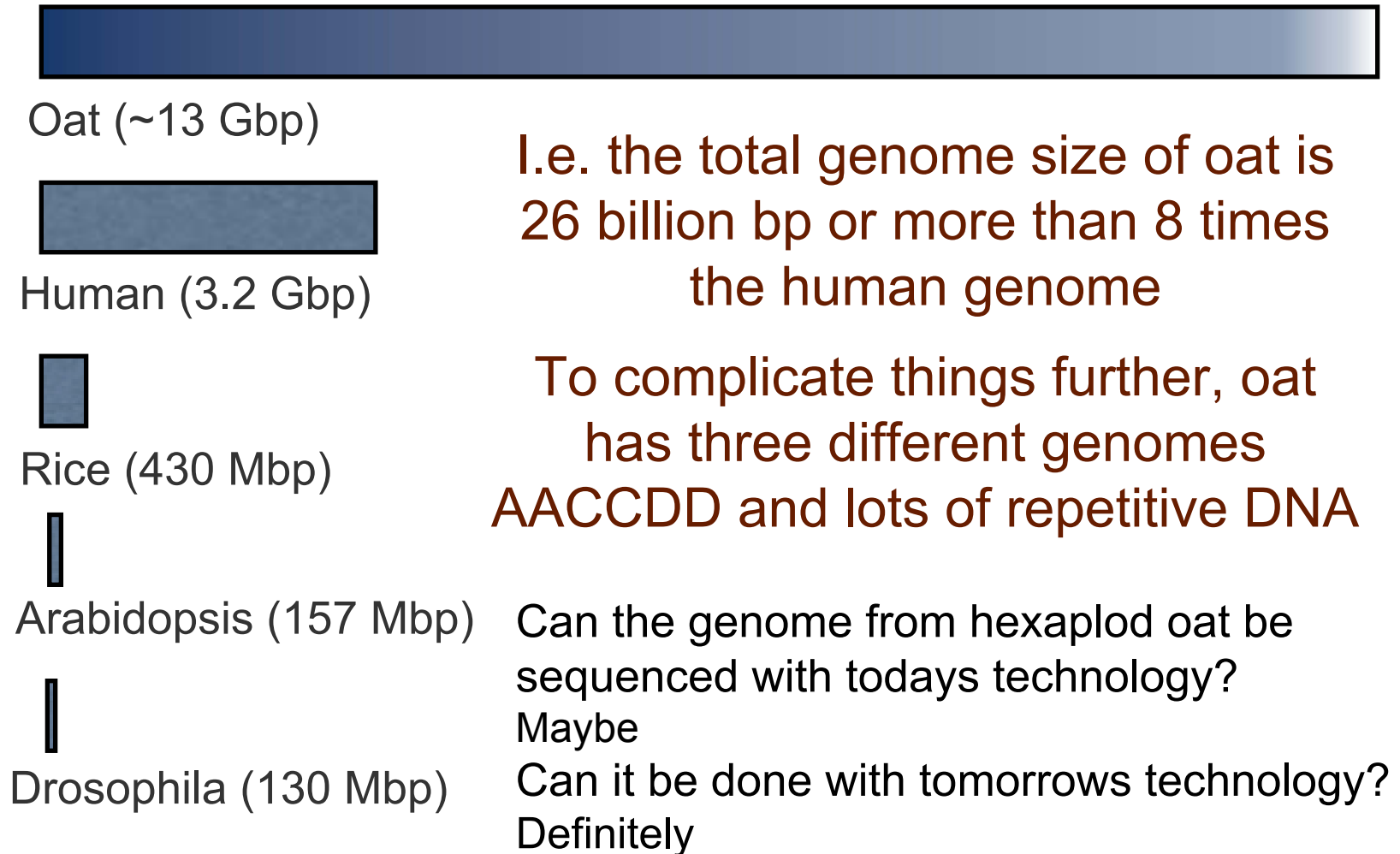


Presently we
have ca 142
million reads

To more efficiently couple mutations
to phenotype it would be good to
have the entire genome sequence
for Belinda

Useful
biological
information

Genome size (1C) in chosen organisms



Coworkers and collaborators (oat project)

- **β-glucan;** Per Sikora (grad stud), Negar Kavooosi (pre grad stud), Dr. Susan Tosh, Canada, Swedish Oat Fiber AB, Aventure AB, CropTailor AB
- **Protein;** Bindu Sunilkumar (grad stud), Prof. Björn Bergenståhl, LU, Dr Lars Nilsson LU, Prof. Rickard Öste, Prof. Ana Racon, Aventure AB
- **Lipids;** Dr Aakash Chawade (researcher), Prof. Thomas Moritz (UPSC, Umeå), Dr. Angeliki Triantafyllou, Oatly AB, Dr Nils-Christian Afseth, Nofima, Bente Kirkhus, Nofima
- **Lignin;** Dr. Vivek Vivekanand, UMB, Norway, Dr. Mikael Larsson (Chalmers, Gothenburg)
- **Sapponins;** Dr. Oswaldo Hernandez (postdoc), Prof. Anders Jonsson (SLU), Tova Andersson, (pre grad stud)
- **Avenanthramides;** Prof Olov Sterner, Narda Blanco, Oswaldo Hernandez
- **Winter oat;** Dr. Aakash Chawade, Dr. Angelica Lindlöf, CropTailor AB
- **Fusarium;** Johanna Olsson CropTailor AB, Per Fahlberg (grad stud) GU, Dr Mats X Andersson, GU