**Carbohydrate microarrays and their use for the identification of molecular markers for plant cell wall composition**

**Running title:** Carbohydrate microarrays for association mapping

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**Abstract:** Genetic improvement of the plant cell wall has enormous potential to increase the quality of food, fibres and fuels. However, the identification and characterisation of genes involved in plant cell wall synthesis is far from complete. Association mapping is one of the few techniques that can help identify candidate genes without relying on our currently incomplete knowledge of cell wall synthesis. Yet few cell wall phenotyping methodologies have proven sufficiently precise, robust or scalable for association mapping to be conducted for specific cell wall polymers. Here we created high-density carbohydrate microarrays containing chemically extracted cell wall polysaccharides collected from 331 genetically diverse *Brassica napus* cultivars and used them to obtain detailed, quantitative information describing the relative abundance of selected non-cellulosic polysaccharide linkages and primary structures. We undertook genome-wide association analysis of data collected from 57 carbohydrate microarrays and identified molecular markers reflecting a diversity of specific xylan, xyloglucan, pectin and arabinogalactan moieties. These datasets provide a detailed insight into the natural variations in cell wall carbohydrate moieties between *B. napus* genotypes and identify associated markers which could be exploited by marker-assisted breeding. The identified markers also have value beyond *B. napus* for functional genomics, facilitated by the close genetic relatedness to the model plant *Arabidopsis*. Together, our findings provide a unique dissection of the genetic architecture that underpins plant cell wall biosynthesis and restructuring.

**Significance statement:** Plant cell wall (PCW) composition determines the nature and quality of many biologically-derived products and therefore is a major target for genetic improvement. However, the identities and functions of many genes involved in PCW synthesis are still not known. Genome-wide association mapping studies (GWAS) are one of the few ways to identify these novel genes. However, collecting precise and quantitative PCW phenotype data at the scale required for GWAS is a significant challenge. Here, we demonstrate for the first time that high-density carbohydrate microarrays can be used as a PCW phenotyping strategy suitable for GWAS. Results presented in this study will aid in the understanding of PCW genetics and crop breeding for improved PCW composition.

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**Introduction**

Plant cell wall composition and structure determines the nature and quality of numerous biological products. These include agronomic traits, such as resistance to pests and diseases, and many of the quality characteristics of biologically-derived products such as natural fibres, timber and food. These in turn have wide reaching impacts on human activities from soil conditioning to human health. Emergent technologies such as lignocellulose-derived biofuels and bio-based polymers could also be made more feasible through modifications in cell wall design ([1](#_ENREF_1)). Consequently, the plant cell wall continues to be a major target for biotechnological improvement.

Marker-assisted selection is a direct and cost-effective way to introduce novel, heritable agronomic improvements into crop varieties and could therefore be used for plant cell wall improvement. Molecular markers provide the genetic tools needed for implementation, enable potentially exploitable genes to be located and help elucidate the genetic mechanisms involved. Recent improvements in genome sequencing have enabled genome characterization, identification of structural rearrangements and high-density linkage maps to be constructed for crop species, such as *Brassica napus*, which have large and complex genomes ([2-4](#_ENREF_2)). Consequently, genome-wide association studies (GWAS) can now be conducted in crop species ([5](#_ENREF_5)).

*Brassica napus* is a good choice for functional genomics, benefiting from its well-studied genetics and its relatedness to the model plant *Arabidopsis* ([6](#_ENREF_6)). As an allotetraploid species with genomes inherited from two, closely related, ancestral species: *B. rapa* and *B. oleracea* (which contribute to the A and C genome portions, respectively), methods needed to identify single nucleotide polymorphisms (SNPs) in orthologous regions within the ancestral genomes have been developed ([3](#_ENREF_3), [7](#_ENREF_7)) and are continually improved ([4](#_ENREF_4)). *B. napus* was also recently used to develop associative transcriptomics where sequence variation, transcript abundance and phenotype are correlated ([7](#_ENREF_7), [8](#_ENREF_8)). The resulting ‘gene expression markers’ (GEMs) have the potential to reveal additional layers of genetic detail, beyond that of traditional GWAS ([7](#_ENREF_7)).

Accurate positioning and identification of tightly linked and robust markers is essential for gene candidate identification. This is particularly important for plant cell wall-related traits, where potential candidates are very common (>10% of the genome)([9](#_ENREF_9)). Precise phenotyping methods are also needed, to prevent the dispersion of genetic signals amongst too many loci.

However, suitably rapid and accurate phenotyping techniques have, until recently, been beyond the reach of cell wall chemists ([9](#_ENREF_9), [10](#_ENREF_10)). GWAS typically require phenotype data to be collected from hundreds or thousands of individuals, which can be difficult to achieve using conventional analytical approaches. Obtaining replication without compromising phenotyping specificity is therefore a formidable challenge. The inherent problems in obtaining necessary replication and comparative analytical data across thousands of samples have led cell wall researchers to either i.) conduct deliberately underpowered GWAS that reveal loci controlling a large proportion of the trait ([10](#_ENREF_10)), ii.) focus on more tractable, but less precise phenotypes to achieve sufficient replication. Examples include the gravimetric determination of bulk polymer fractions ([11](#_ENREF_11), [12](#_ENREF_12)), indirect determination of composition using pyrolysis ([13](#_ENREF_13)) or focussing on a particular product, such as sugar release following saccharification ([14](#_ENREF_14), [15](#_ENREF_15)). These mapping project successes illustrate the clear potential of GWAS to elucidate relevant genetic targets, but also highlight the requirement for greater phenotyping specificity or increased replication ([9](#_ENREF_9)).

Some high-throughput cell wall phenotyping approaches such as Fourier transform infra-red screening ([16](#_ENREF_16), [17](#_ENREF_17)), OLIgo Mass Profiling (OLIMP) ([18](#_ENREF_18)) and carbohydrate microarray-based ‘CoMPP’ profiling ([19](#_ENREF_19), [20](#_ENREF_20)) are now available, but have yet to be deployed with the scale and precision required for association mapping. Hence, these methods have generally been used for intervention-based or comparative studies, which do not require the same degree of quantification or replication. Recently, Lin *et al.* ([21](#_ENREF_21)) used a range of high-throughput cell wall profiling methodologies to collect cell wall phenotype data from 30 samples at various stages of development and correlated these against the transcript abundance of 67 putative cell wall genes. This information will help assign functions to genes known to be involved in cell wall synthesis. GWAS have the potential to take this further to identify novel candidates in addition to those that are already known. However, prior to the present study, high-throughput phenotyping technologies have not been sufficiently precise and integrated to collect large-scale datasets suitable for GWAS without considerable effort.

Here we developed high-density carbohydrate microarrays suitable for association mapping (**Fig. 1A**). Particular care was taken to produce precise, quantitative and comparable data while minimising the accumulation of technical errors that might hinder precision. Using this data, we aimed to elucidate areas of the transcriptome that are most closely associated with variation in cell wall polymer abundances in mature stem tissue of *B. napus* using associative transcriptomics ([4](#_ENREF_4), [7](#_ENREF_7), [8](#_ENREF_8), [22](#_ENREF_22)).

We chemically extracted four carbohydrate-rich fractions from 331 diverse *B. napus* genotypesin duplicate, following common plant cell wall extraction regimes ([23](#_ENREF_23)) adapted for screening, and printed them as carbohydrate microarrays. Although chemical extraction tends to release fractions rich in certain carbohydrates, the extracts are not pure ([23](#_ENREF_23)). Rather, each fraction contains a mixture of carbohydrate moieties derived from various polymer classes, which vary in solubility depending on their chemistry and interaction with other components ([23](#_ENREF_23)). Chemical or enzymatic extraction methodologies and downstream chromatographic procedures could be selected to isolate polymers more precisely or less destructively than those used here.

Here we used ammonium oxalate to mainly release polysaccharides bound by metal ions. Sodium carbonate was then used to de-esterify cell wall components, releasing mainly pectins held by weak-ester linkages and to stabilise more sensitive polysaccharides to β-eliminative degradation ([24](#_ENREF_24)). Further extraction with 1M and 4M KOH was used to solubilise predominantly xylans. Using half-gram portions of cell wall material ensured a reliable datum to which all samples were comparable and minimised sample heterogeneity ([25](#_ENREF_25)). Plate-based liquid handling robotics for soluble extracts minimised technical errors. To obtain a high-throughput quantitative measurement of selected carbohydrate moieties extracts were printed as high-density glycan microarrays, containing dilution series of each extract (**Fig. 1B**). These were probed with monoclonal antibodies (mAbs) raised to different cell wall components ([26](#_ENREF_26), [27](#_ENREF_27)) (**Fig. S1-4**). Labelling with a secondary antibody conjugated to a fluorophore enabled detection.

Most carbohydrate microarrays use chromogenic methods of detection, probing samples that have been diluted to obtain an unsaturated feature ([19](#_ENREF_19)). Here we used a fluorescent probe for detection, which increased sensitivity and specificity, but meant that no single dilution could bring all measurements of all samples into the linear range (**Fig. 1C**). We therefore printed two, eight-point dilution series of each extract and modelled the concentration-related change in fluorescence using non-linear regression. From the resulting best-fit lines, we used a threshold method of quantification analogous to that of quantitative PCR, to interpolate the dilution required to obtain a feature with a set fluorescence in the linear range. The inverse of the dilution was used to derive a nominal relative abundance of each epitope relative to the original sample weight for each extract. The relative abundance measurements collected from each extract were averaged for each cultivar and expressed relative to an average cultivar (**Fig. S5-8,** example: **Fig. 1D**). Methodological improvements, such as non-contact printing of arrays, might improve precision further. Less variable data might also be obtained using liquid-based ELISA methods, but this would be logistically challenging and more costly to achieve.

Here, microarray-based abundance measurements, suitable for association mapping, were made for 57 slides (Example: **Fig. 1C**, images: **Fig. S5-8,** data: **Table S1**). Antibodies raised to common cell wall polysaccharides (including xylan, xyloglucan, homogalacturonan, rhamnogalacturonan or arabinogalactan) allowed the relative quantification of specific epitopes, by exploiting their varying binding specificities ([23](#_ENREF_23), [27](#_ENREF_27)). With over 200 carbohydrate-directed mAbs ([26](#_ENREF_26), [27](#_ENREF_27)) and multiple cell wall extraction methods to choose from, the epitopes targeted in this study are only a small selection of those present in the wall. The aim of this work was not to provide an exhaustive overview of cell wall moieties but to assess the suitability of high-throughput cell wall glycomics as a phenotyping strategy for association mapping – targeting a selection of linkages likely to differ in their extractability, specificity, and underlying genetics.

To identify arrays likely to show similar marker associations after mapping, we correlated the mean relative abundance data collected from each array and ordered them by hierarchical clustering (**Fig.** **2**). The array data broadly clustered into mAbs that recognised either XG or xylan moieties, the main hemicelluloses found in dicot primary and secondary cell walls ([28](#_ENREF_28)) (**Fig. 2**). Further clustering of the datasets, within these groups, conformed to subtle differences related to polymer solubility or mAb specificity. For example, cultivars with stems rich in one xylan epitope also tended to contain more of other xylan epitopes, but those extracted using 1M KOH could generally be distinguished from those released by 4M KOH (**Fig. 2**). Conversely, some mAbs such as CCRC-M78 and CCRC-M92 which recognise Arabinogalactan-4 moieties ([23](#_ENREF_23), [27](#_ENREF_27)), showed similar genotypic differences in relative abundance, irrespective of the extract the probed. Therefore, one might expect data from these arrays to produce similar patterns of marker association with a common genetic basis. Similar inferences may be made for other epitopes in different extracts, which are likely to share similar genetics (**Fig. 2**).

High-density carbohydrate microarrays permitted us to obtain detailed and biologically-relevant data pertaining to the relative abundance of complementary and contrasting cell wall epitopes, in parallel, at the scale required for GWAS. To assess the suitability of these datasets for association mapping, we mapped all 57 datasets using associative transcriptomics ([4](#_ENREF_4)). Utilising the unique ability for associative transcriptomics to identify molecular markers based on both SNP-based variants and transcript abundance (GEMs), we also identified putative transcription-based regulators of cell wall composition (**Fig. S9-65**).

As proof of concept, we illustrate the deployment of the method to dissect the genetic basis for xylan synthesis and branching, which is already largely understood ([29-37](#_ENREF_29)), by exploiting the differing binding specificities of two mAbs, CCRC-M139 and CCRC-M150 (**Fig. 3**).

Dicot-derived xylan can be broadly divided into two classes defined by the most common substitution patterns ([36](#_ENREF_36)). ‘Major domain’ xylan is decorated with glucuronic acid (GlcA) residues distributed sparsely but evenly along the backbone (approximately every 8-10 Xyl residues)([37](#_ENREF_37)). ‘Minor domain’ xylan is decorated with GlcA more frequently and unevenly (typically every 5 residues), theoretically altering its interactions with other components ([36](#_ENREF_36)).

Antibodies that adhere to both xylan domains, irrespective of branching patterns, should reveal variations in overall xylan abundance (**Fig. 3A**). In contrast, the abundance of ‘major domain’ xylan could be indirectly quantified using mAbs that exclusively recognise sparsely-substituted sections, thereby implicating the genes that produce these patterns (**Fig. 3A**).

Although the exact epitopes recognised by many carbohydrate-directed mAbs are not known, CCRC-M150 has been shown to bind to unbranched xylan oligosaccharides > 4 Xyl residues in length, or arabinoxylan oligosaccharides > three Xyl residues in length irrespective of substitution patterns ([38](#_ENREF_38)). Therefore, CCRC-M150 should identify markers in areas of the transcriptome common to both ‘major’ and ‘minor’ xylan domains, for example those involved in xylan backbone synthesis. By contrast, CCRC-M139 binds specifically to longer unsubstituted Xyl oligomers > six Xyl residues in length (**Fig. 3A**). Binding can be prevented by arabinose substitutions ([38](#_ENREF_38)). CCRC-M139 should therefore preferentially bind to ‘major domain’ xylan domains, implicating genes that alter the relative abundance of these long unbranched sections when mapped. We therefore mapped the relative abundance of epitopes recognised by CCRC-M150 and CCRC-M139 in the 4M KOH fractions which are likely to contain a greater concentration of ‘major domain’ xylan, as higher alkali concentrations are required to disrupt hydrogen-bonds formed with other components ([37](#_ENREF_37)).

For CCRC-M150, we identified two hemi-SNP markers of genome-wide significance (Bonferroni-corrected, *p* < 0.0000001, **Table S2**) located on Chr. A1, flanking a *B. napus* orthologue of IRREGULAR XYLEM 14 (IRX14) (Chr. A1: **Fig. 3B, left**. Full plot: **Fig. S61**), shown to be important for xylan backbone synthesis in many species ([29-35](#_ENREF_29)). Variations in the most highly associated marker (Cab020414.1:492) revealed a common SNP variant (91/294 cultivars) that coincided with a *ca.* 25% reduction in CCRC-M150 binding (**Fig. 3C, left**). Selection for the allele associated with lower xylan abundance could be used to reduce xylan content in stem tissue by marker-assisted breeding. Potential industrial benefits for xylan reduction include the tailoring of biomass to produce specific products or reduction of recalcitrance for biorefining ([1](#_ENREF_1)).

In contrast, we identified seven SNPs of genome-wide significance associated with CCRC-M139 binding (**Table S2**). Despite probing identical extracts, these markers were located in different parts of the transcriptome compared to those associated with CCRC-M150 binding (Chr. A1: **Fig. 3B, right**. Full plot: **Fig. S57**). All of these markers were located near to *B. napus* orthologues of GLUCURONIC ACID SUBSTITUTION OF XYLAN 1 (GUX1) located at homeologous positions on Chr. A1 and C1 (Cab008614.1 and Bo1g116390.1, **Fig. S57**). In *Arabidopsis*, GUX1 exclusively decorates glucuronoxylan with GlcA every 8-10 Xyl residues, to produce sparsely-branched sections of ‘major domain’ xylan which cannot receive further GlcA substitutions ([36](#_ENREF_36)). The positions of these markers are therefore consistent with the specificity of CCRC-M139, which must bind to long unsubstituted sections determined by GUX1. Specific alleles at these loci, such as a T allele at Cab008599.2:210, could be used to select germplasm with a greater proportion of sparsely branched xylan (**Fig. 3C**, right). Reducing xylan branching may be favourable to modify extractability during industrial processing, reducing polymer heterogeneity ([39](#_ENREF_39)) and influencing the interactions between xylan and other cell wall components ([37](#_ENREF_37)).

The coincidence of markers surrounding *B. napus* orthologues of IRX14 and GUX1 are not only consistent with known specificities of the mAbs used, but also implicate the main genetic players in this process. Overall, this demonstrates that high-density quantitative glycan microarrays, used in conjunction with association mapping, can detect pertinent variations related to plant cell wall genetics.

Additional associations provide insights into the genetic architecture underpinning cell wall composition of other polymer linkages, with less well known genetics (**Fig. S9-65**), which are influenced by a combination of genetic and methodological factors distinct to each array. Potential genetic factors include the number and relative contributions of different loci contributing to a single trait. For example, unlike the example given above which implicates genes located at few precise loci, a large number of genes are involved in the synthesis and remodelling of xyloglucan ([40](#_ENREF_40)). The resulting marker associations for these traits are therefore less distinct and dispersed across more loci (**Fig. S37-39, S48-56**).

Methodological differences such as tissue and cell-type variations within each sample, the chemicals used for extraction, the exact specificity of each mAb and accuracy of quantification also alter genetic signals to varying degrees. For example, obtaining precise quantification for mAbs that recognise particularly rare linkages can be difficult. This can be seen for CCRC-M151 binding to the 4M KOH fraction; although it has a similar binding specificity to CCRC-M139, only one tail of the distribution could be accurately quantified above the limit of detection (**Fig. S8**).

Nevertheless, for many epitope-based traits, marker associations clearly implicate regions of the transcriptome harbouring novel gene candidates worthy of further investigation which will form the basis of future studies. Particularly interesting examples include those related to MAC204 binding to oxalate-extracts (**Fig. S18**) which show SNP marker associations at homeologous positions on Chr. A3 and C3. These, together with a single associated GEM marker located on Chr A3 (A03\_005776413\_005778886), strongly implicate Cab016526.3, a *B. napus* ortholog of BETA-GALACTOSIDASE 4 (AT5G56870), as the most likely candidate gene.

Similarly, all slides probed with mAbs that identify Group-4 Arabinogalactans showed increases in GEM associations surrounding Bo3g004890.1, a *B. napus* ortholog of ALPHA-GALACTOSIDASE 1 (AT5G08380) on Chr C3, despite probing different extracts (oxalate and carbonate) with two different mAbs (CCRC-M78 and CCRC-M92)(**Fig. S21, S22, S35, S36**). These candidate genes are highly likely to alter the abundance and composition of arabinogalactan moieties in mature dicot cell walls and illustrate the deployment of this method to identify novel gene candidates involved in cell wall biosynthesis.

Molecular markers highlighted in this study will provide considerable assistance to those attempting to identify the roles of particular genes by intervention-based approaches, enhanced by the close relatedness between the model plant *Arabidopsis* and *Brassica* species ([6](#_ENREF_6)). Marker-assisted breeding could be used to generate germplasm with altered cell wall chemistry, using the molecular markers we have identified. Similar GWAS could be conducted for other abundant or commercially important cell wall types, such as those in edible tissues, or those with different cell wall architecture, such as monocotyledonous plants, or other organisms with glycan-rich cell walls. More generally, quantitative high-density carbohydrate microarrays will prove a useful phenotyping strategy, providing data suitable for use in association mapping and greatly expand our knowledge of cell wall genetics.

## Materials and Methods

**Materials.** All *B. napus* straw accessions were grown in 5 L plant pots at randomised locations in a 30 m x 7 m polytunnel located at the University of Nottingham, Sutton Bonington Campus, Leicestershire (+52° 49' 56.65", -1° 14' 57.73")([41](#_ENREF_41)). Stems were left to dry in their pots and basal stem material from 2 cm above the hypocotyl to ca. 60 cm above the ground was collected from each accession at maturity (20-22 Aug 2014). Stem material from individual plants were then milled using a Cyclone Mill TWISTER (Retch®, Germany) fitted with a 0.5 mm screen to produce fine, uniform powders. After milling, circa. 84%, 61% and 24% of the sample by weight was able to pass a 250, 150 and 53 µm sieve, respectively. In total, 475 stem accessions derived from 330 different cultivars were analysed in duplicate. Milled straw (ca. 3.5 g) from each accession was rinsed in 50 mL ethanol (≥ 99.8%, 1 h, RT), followed by acetone (≥ 99.8%, 1 h, RT), separating solid and liquid components by centrifugation (4000 RPM, 15 min, Eppendorf 5810R with A-4-81 rotor) and discarding the supernatant. The pellets surface area was increased with a spatula, and acetone left to evaporate (3 d, RT), producing a cold alcohol/acetone insoluble residue (AIR).

**Sequential extraction of cell wall polysaccharides.** In total, 950 AIRs derived from 475 stem accessions collected from 330 different *B. napus* cultivars were sequentially extracted. Controls containing a mixture of AIRs from ten randomly selected accessions were also included with each set of 88 to observe any plate-to-plate variations (extraction control - A12). A bulk extract containing 2 g of combined AIR, 40 mL of extractant, was also included with each plate (quantification control – position B12). AIR from each accession (0.5 g) was weighed into 15 mL polypropylene tubes in duplicate on a three-decimal-place balance and order randomised before extraction. Each sample was mixed with ammonium oxalate (10 mL, 50mM) and extracted overnight (RT). A sample of each supernatant (1 mL) after centrifugation was loaded into two 96-well 2 mL deep-well polypropylene plates (Nunc®, Roskilde, Denmark) (positions A1 to H11) using an Andrew™ Liquid Handling Robot (Andrew Alliance, Switzerland). The remaining supernatant from each sample was discarded and the pellet washed thrice in ddH2O. This process was repeated using Na2CO3 (50mM) + NaBH4 (0.5% w/v), 1M KOH + NaBH4 (1% w/v) and 4M KOH + NaBH4 (1% w/v). Pellets were not washed between collection of the 1M and 4M KOH fractions ([23](#_ENREF_23)). An anti-foaming agent, 1-Octanol (10 µL), was added to each Na2CO3, 1M KOH and 4M KOH extract before gradual neutralisation to pH 5 with glacial acetic acid (9, 50 and 200 µL respectively) using a multi-channel pipette and stored at -20 °C.

**Relative quantification using carbohydrate microarrays.** Cell wall extracts held in 96-deep-well plates were mixed (20 aspiration and dispense cycles of 80 µL) and sub-samples transferred to 384-deep-well plates (80 µL) using a Tecan Freedom Evo® 100 workstation fitted with a 96-well MultiChannel Arm (MCA96) with disposable tips (Tecan, Männedorf, Switzerland). Seven sequential, two-fold dilutions of each sample (50% to 0.78% original) were made across separate 384-deep-well plates (final volume 20 µL). Samples were printed onto FAST Slides (Whatman) using a bespoke Stanford-designed microarray printer (http://cmgm.stanford.edu/pbrown/mguide/) equipped with SMT-S75 silicon printing pins (Silicon Microarray™, CA, USA). Duplicate 96 x 96 square arrays consisting of 75-110 µm square features at 175 µm intervals were printed on each slide in 20 slide batches, at elevated humidity (> 60% RH, 18-23°C). After printing, slides were stored over P2O5 before mAbs probing. Slides were blocked with 1% non-fat milk in 0.1 M Tris-buffered saline (1% MTBS, pH 7.6, 2 h). Arrays were incubated overnight with CCRC, JIM and MAC-series primary mAbs (50 µL, sourced from Carbosource, GA, USA), diluted 500 times in 0.1% MTBS with 0.01% thiomersal added to prevent microbial contamination. Slides were rinsed at least thrice in 0.1% MTBS (50 mL), and incubated with a secondary antibody (Anti-Rat or Anti-Mouse IgG produced in goat conjugated to Alexa Fluor® 555, diluted to 5 µg/mL in 0.1% MTBS, 1h). The secondary antibody was removed by rinsing five times in 0.1% MTBS (50 mL ea.) and dried by centrifugation (500 RPM, 3 min, RT). Slides were read at an excitation wavelength of 532 nm using an [Axon 4200AL microarray scanner](http://www.labx.com/item/axon-4200al-microarray-scanner/1048704) and analysed using GenePix Pro 6.1 software. PMT gain was adjusted to maximise the dynamic range across each array (Maximum fluorescence >66,000). Data were fitted to the Gompertz equation and the dilution required to generate a mid-range fluorescence calculated using R (SSgompertz, http://www.r-project.org/). Broad exclusion criteria were set to remove samples which were outside the limits of detection. This included either sample features which were not discernible from the background (blanks) or where the most extensive dilution used here (1/128) was not sufficient to bring the feature fluorescence into the linear range. The data were then aggregated by calculating the mean relative abundance of each epitope (1/dilution factor) for each genotype.

**SNP detection, gene expression and associative transcriptomics.** Mapping and associative transcriptomics was conducted using previously described methods and scripts ([3](#_ENREF_3), [7](#_ENREF_7), [42](#_ENREF_42), [43](#_ENREF_43)), with minor modifications. For full details, please see **SI Materials and Methods**. In total, 9839 simple SNPs and 246558 hemi SNPs, along with 53889 significantly expressed coding DNA sequence models (> 0.4 mean RPKM) were used for associative transcriptomics.

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**Figure Legends**

**Fig. 1** The use of high-density carbohydrate microarrays as a phenotyping method for association mapping. **A**. An overview of phenotyping strategy: We collected stem tissue from 475 accessions, derived from 331 cultivars, and sequentially fractionated them in duplicate, releasing four glycan-rich fractions (ca. 3800 extracts in total). **B**. Microarray layouts: Two-fold dilution series (*n* = 8) of each cell wall extract were printed together, as duplicate 96 x 96 square arrays consisting of ca. 100 µm square features. Each slide contained circa. 15,500 features extracted using the same conditions. We probed each extract using 19 glycan-specific antibodies (76 slides in total, Images: **Fig. S1-4**). **C**. Example of fluorescence measurements collected from a single slide, grouped by dilution: Boxes indicate the interquartile range (IQR) and the central line indicates the median fluorescence for these features. Whiskers and points indicate data 1.5 x IQR inside or outside of the quartiles, respectively. The boxplots show that no single dilution, even in the middle of the range (diluted 32 times), would bring all fluorescence measurements of all samples into the linear range. Therefore, to obtain accurate quantification for all samples, we used non-linear regression to fit best-fit lines to each dilution series. From these lines, we interpolated the dilution that would be required to produce a feature with a set fluorescence within the linear range. The inverse of the dilution was then used to derive a nominal relative abundance of each epitope relative to the original sample weight. **D**. Relative abundance measurements taken from the same genotype were averaged and their abundance expressed relative to the mean cultivar: An example histogram showing the distribution in mean-centred relative abundance of a single epitope between 331 cultivars is shown here. In total, relative quantification was obtained for 57 slides (**Fig. S5-8**), amassing data collected from over one million data points. These data were used for association mapping.

**Fig. 2** Correlation matrix comparing the relative abundance measurements for 330 different *B. napus* cultivars collected from 56 arrays. The heat-map displays pair-wise spearman’s rank correlation coefficients between data collected from each array (key located in the top left-hand corner), ordered by hierarchical clustering by the complete linkage method (dendrograms). For legibility, array identities including the extract and primary antibody (1-57) are listed on the right-hand side. The left-hand colour key depicts the chemical used for extraction and the uppermost colour key depicts the general polymer class commonly associated with each primary antibody following Pattathil *et al*. ([23](#_ENREF_23), [27](#_ENREF_27)). Please note, the binding specificities of some mAb are not exclusive to a single polymer class. Correlated epitopes are more likely to have a common genetic basis and are likely to produce more similar GWA profiles after mapping.

**Fig. 3** As proof of concept, we mapped the relative abundance of two epitopes recognised by two xylan-specific mAbs, CCRC-M150 and CCRC-M139, in a xylan-rich extract (4M KOH). **A.** Likely differences in antibody binding to xylan; Although the precise binding specificities of these mAbs are not known, CCRC-M150 is thought to bind to most xylan structures, whereas CCRC-M139 preferentially binds to long unsubstituted sections > six Xyl residues in length, at least for arabinoxylan oligomers known to differ in composition ([38](#_ENREF_38)). **B.** Manhattan plots which show the degree of association (y-axis) of all SNPs located on chromosome A1 (x-axis) with the genotypic variation in CCRC-M150 or CCRC-M139 binding to the 4M KOH extracts: SNPs with a higher -log10(P) value show a greater statistical association with each trait. Those above the Bonferroni threshold (grey dotted line) pass a conservative level of genome-wide statistical significance (*p* = 0.05/256598 markers). Hemi-SNPs and Simple-SNPs (not assigned / assigned to a specific ancestral genome, respectively) are coloured light and dark blue, respectively. SNPs associated with genome-wide significance to the variations in CCRC-M150 and CCRC-M139 binding to the 4M KOH extracts, coincide with regions harbouring orthologues of *Arabidopsis* IRX14 and GUX1 respectively (x-positions shown in red). These marker associations are consistent with the respective substitution patterns that these mAbs are thought to recognise. The most highly associated markers on chromosome A1 have been indicated for both traits (grey text). **C.** Boxplots showing variations in relative mAb binding to the 4M KOH extracts, grouped by the alleles at these significantly associated SNP loci: The number of cultivars tested with a particular allele and the mean relative abundance of each epitope are written in each box in black and red respectively. For example, most cultivars (*n* = 188) had a T allele at SNP ‘Cab020414.1:492’. Cultivars with a G allele at this position (*n* = 91) tended to contain less of the epitope recognised by CCRC-M150 in the 4M KOH extract.