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Mapping and validation of powdery mildew resistance loci from spring wheat cv. Naxos with SNP markers

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Abstract

Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* is a major wheat disease in maritime and temperate climates. Breeding for race-non-specific or partial resistance is a cost-effective and environmentally friendly disease control strategy. The German spring wheat cultivar Naxos has proven to be a good source for partial resistance to powdery mildew. The objectives of the present study were to map the resistance loci in Naxos with use of high-density SNP markers in the Shanghai3/Catbird x Naxos inbred line population and validate the results in a different genetic background; Soru#1 x Naxos. Both populations were genotyped with the Illumina iSelect 90K wheat chip, and integrated linkage maps developed by inclusion of previously genotyped SSR and DArT markers. With the new linkage maps, we detected a total of twelve QTL for powdery mildew resistance in Shanghai3/Catbird x Naxos, of which eight were derived from Naxos. Previously reported QTL on chromosome arms 1AS and 2BL were more precisely mapped and the SNP markers enabled discovery of new QTL on 1AL, 2AL, 5AS and 5AL. In the Soru#1 x Naxos population, four QTL for powdery mildew resistance were detected, of which three had resistance from Naxos. This mapping verified the 1AS and 2AL QTL detected Shanghai3/Catbird x Naxos, and identified a new QTL from Naxos on 2BL. In conclusion, the improved linkage maps with SNP markers enabled discovery of new resistance QTL and more precise mapping of previously known QTL. Moreover, the results were validated in an independent genetic background.

Keywords: Wheat, Illumina 90K SNP Chip, Powdery mildew, QTL mapping.

Key message: QTL for powdery mildew resistance were discovered and validated in two wheat recombinant inbred line populations utilising maps with SNP, SS and DArT markers.

Author contributions:

Susanne Windju analysed and scored the SNP markers from the Illumina 90K SNP Chip in Genome Studio, performed linkage mapping in Join Map and QTL mapping in MapQTL, analysed field data in the RIL Soru#1 x Naxos, wrote the manuscript.

Keshav Malla performed field work, data collection and analysis.

Tatiana Belova performed linkage mapping in MST map, assigned markers to chromosomes.

Robert C. Wilson supervised the work, reviewed the manuscript.

Jon Arne Dieseth executed the field design and experiment, reviewed the manuscript.

Muath Alsheikh obtained funding, supervised the work, edited the manuscript.

Morten Lillemo obtained funding, designed and led the project, designed and performed fieldwork, supervised the work, edited the manuscript.

Introduction

Powdery Mildew (PM) caused by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici (Bgt)*, is one of the devastating diseases of wheat in areas with maritime and temperate climates (Bennett, 1984). It can cause significant yield losses ranging from 13% to 34%, but if the disease attacks are severe to the flag leaf during the heading and grain filling stage, losses can reach 50% (Alam et al., 2013; Griffey et al., 1993). Powdery mildew infection and disease development have been favoured due to widespread use of irrigation, semi-dwarf cultivars and nitrogen fertilizers (Bennett, 1984; Selter et al., 2014). Chemicals are extensively used to control the disease and maintain high yields when susceptible cultivars are grown. Breeding of resistant cultivars is a more economical and environmentally safe disease control strategy (Petersen et al., 2014; Worthington et al., 2014).

Two main types of resistance to powdery mildew are generally recognized: race-specific and race-non-specific. Race-specific resistance is qualitative, and also called vertical, or seedling resistance and mediated by single major race-specific (*Pm*) genes of relatively large effects (Bennett, 1984). This type of resistance works through recognition of the pathogen in a gene- for- gene relationship (Flor, 1955). Race-specific resistance gives complete protection to specific races of pathogens and are, usually, effective only against some isolates of powdery mildew, but ineffective to others (McDonald et al., 2002). Due to the high vulnerability to genetic changes in the pathogen, new virulent races can quickly evolve to overcome single race-specific resistance genes, resulting in short durability of resistance (Hsam et al., 2002; McDonald et al., 2002).

Race-non-specific resistance is quantitative or partial (Hautea et al., 1987) and controlled by several genes with major or minor effects. This type of resistance is also known as 'adult-plant resistance' (APR) (Gustafson et al., 1982) or 'slow mildewing'(Roberts et al., 1970) as several resistance genes work together to reduce the infection efficiency and retard growth and reproduction of the pathogen, especially in adult plants (Shaner, 1973). Breeding of wheat cultivars with partial resistance to powdery mildew has been suggested as a more promising and sustainable strategy to control this disease. It may be difficult to identify and select for partial resistance in the field, especially in the

presence of race-specific resistance genes that mask the effect of race-non-specific resistance during field selection (Keller et al., 1999; Lillemo et al., 2010). In such situations, molecular markers offer the opportunity to select directly for the presence of genes for partial resistance, once they have been mapped and validated.

Since the first dominant resistance gene *Pm1* was described in 1953 (Pugsley et al., 1953) more than 77 powdery mildew resistance genes or alleles at 49 loci (*Pm1-Pm54)* have been catalogued and assigned to specific chromosomes and chromosome arms in common wheat (Hao et al., 2015; Ma et al., 2015). Lillemo et al. (2008) described two race-non-specific genes, *Pm38 and Pm39*, which exhibit strong partial resistance to powdery mildew, and are pleiotropic to the rust resistance genes *Lr34/Yr18 and Lr46/Yr29*, respectively. Furthermore, the race-non-specific powdery mildew resistance gene *Pm46* was found to be pleiotropic to the rust resistance locus *Lr67/Yr46* (Herrera-Foessel et al., 2014; Moore et al., 2015)

During the past two decades, different types of molecular markers have been used to localize powdery mildew resistance genes in the wheat genome (Li et al., 2014). More recently, single nucleotide polymorphisms (SNPs) have gained preference in genetic mapping studies. These markers are abundant, co-dominant and evenly distributed across the genome. SNPs can be generated in a high-throughput and cost- effective manner, which makes them an ideal marker system (Colasuonno et al., 2014; Gupta et al., 2008). In recent years, high- density 9K SNP chip and 90K SNP chip platforms have been developed for wheat (Cavanagh et al., 2013; Wang et al., 2014). These high-density genotyping arrays further enhance the development of SNP marker resources for wheat breeding and improve the construction of high-resolution genetic maps for understanding complex traits.

The spring wheat cultivar Naxos shows highly effective partial resistance to powdery mildew in the field while being susceptible at the seedling stage. With its adult plant resistance this cultivar is therefore a valuable source for partial and more durable resistance against powdery mildew (Lillemo et al., 2010; Lu et al., 2012).

A QTL mapping study using SSR and DArT markers in the mapping population Shanghai3/Catbird x Naxos performed by Lu et al. (2012) revealed several important powdery mildew resistant QTL from Naxos. A highly significant QTL originating from Naxos was detected on chromosome 1AS, in the same area as the *Pm3* locus. Naxos is, however, known to not carry any race-specific gene for resistance to powdery mildew. Moreover, it lacks the *Pm3* gene based on the UP3B/UP1A and Pm3MF/Pm3ER1 markers (Lu et al., 2012; Tommasini et al., 2006). The QTL with resistance from Naxos revealed on 1AS is therefore likely due to a gene for race-non-specific resistance. This

QTL was reported to explain up to 35% of the phenotypic variance in some environments in the study. Another major QTL from Naxos was detected on 2DL and two minor QTL on 2BL and 7DS. In addition, QTL with resistance from Shanghai3/Catbird were detected on chromosome arms 1RS, 2DLc, 6BL and 7AL by Lu et al. (2012).

The main objectives of the present study were to detect new QTL for powdery mildew resistance derived from the resistance source Naxos, and to confirm and more precisely map powdery mildew resistance QTL previously detected in the study performed by Lu et al. (2012). In addition, the study aimed at validating the detected QTL in another genetic background and to identify tightly linked SNPs for powdery mildew resistance breeding. By utilising the Illumina iSelect 90K wheat SNP Chip (Wang et al., 2014), marker maps with SNP markers were developed and incorporated with previously genotyped SSR, DArT and gene-specific markers, to achieve high-density marker-maps for QTL - and associated SNP - detection.

Materials and Methods

Plant Material

Two populations of recombinant inbred lines (RIL) in generation F_6 developed by single seed descent were utilized in the study: 177 lines from the cross Shanghai3/Catbird (SHA3/CBRD) x Naxos and 131 lines from the cross Soru#1 x Naxos. Naxos is a German spring wheat developed by Strube GmbH & Co.KG from the cross 'Tordo/St. Mir808-Bastion/Minaret'. Naxos exhibits high levels of partial resistance to powdery mildew at the adult plant stage (Lu et al., 2012). SHA3/CBRD is a breeding line developed at CIMMYT with the pedigree "Shanghai3//Chuanmai 18/Bagula" and selection history "-0SHG-6GH-0FGR-0FGR-0Y". SHA3/CBRD has been shown to be moderately susceptible to powdery mildew, carrying the *Pm8* resistance allele on 1RS and the *Pm3* haplotype on 1AS, but none of the *Pm3a-g* alleles (Lu et al., 2012; Tommasini et al., 2006). Soru#1 is a synthetic hexaploid derived wheat line (AABBDD). It was developed by CIMMYT from the cross 'SABUF/5/BCN/4/RABI//GS/CRA/3/*Ae. tauschii* (190)'. It is highly susceptible to powdery mildew.

Seedlings were grown in the greenhouse in Ås, Norway and genomic DNA was extracted from fresh young leaves of parents, F_6 RILs, and controls using the DNeasy plant DNA extraction kit (Qiagen).

Field evaluation

SHA3/CBRD x Naxos

For the SHA3/CBRD x Naxos population, powdery mildew disease resistance data from Lu et al. (2012) was utilized. The evaluation of powdery mildew resistance had been performed at two locations in Norway in 2008, 2009 and 2010; Vollebekk research farm at the Norwegian University of Life Sciences, Ås (59°N, 90 m above sea level) and Staur research farm close to Hamar (60°N, 153 m above sea level). Both locations experience natural epidemics of powdery mildew, but are characterized by different *Bgt* virulence compositions (Skinnes, 2002). In addition, the population had been evaluated for powdery mildew resistance at three locations in China; In 2009 at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing (32°N, 15 m above sea level), Jiangsu province and in 2010 at the Chinese Academy of Agricultural sciences (CAAS), Beijing (39°N, 43.5 m above sea level), and at CAAS Cotton Research Institute, Anyang (36°N, 70-80 above sea level), Henan province.

Soru#1 x Naxos

For the Soru#1 x Naxos population, powdery mildew disease severity was evaluated at Vollebekk research farm in 2012, 2013 and 2016, and at Staur research farm in 2013.

Field trials were conducted in hill plots using an alpha lattice block design (12 plots per block) with two replications in each experiment. Lines were planted with 50 cm between plots and 40 cm between each row. The susceptible line Avocet-S was planted as spreader rows surrounding the nurseries, and the moderately resistant cv. Bastian was planted as a barrier next to the experimental plots.

Powdery mildew disease severity was assessed on the whole canopy basis as the percentage of leaf area infected, using a modified Cobb scale (0 to 100% infected leaf area) (Peterson et al., 1948). The disease severity was scored two to three times with the first score being made when the most susceptible lines had reached about 50% - 70% severity, and then repeated at weekly intervals until the powdery mildew ceased to develop on the most susceptible lines.

Statistical analysis

For the Soru#1 x Naxos population, phenotypic data of powdery mildew severity was analysed using the software MINITAB 17 (Minitab, 2010). Average percentage of powdery mildew from different dates was calculated. Analysis of Variance (ANOVA) was conducted using general linear model (GLM) to determine differences in mildew scores

among the F_6 lines, and heritability of the phenotypic traits for single environments (h^2) was calculated in Agrobase Generation II from Agronomix Software Inc.

Linkage mapping

The Soru#1 x Naxos and SHA3/CBRD x Naxos populations were genotyped with the Illumina iSelect 90K wheat SNP Chip (Wang et al., 2014). Analysis and scoring of the genotype results for each population was performed manually for every SNP marker with the software Genome Studio Genotyping Module v1.0 from Illumina.

In both populations, markers scored as polymorphic were used for constructing linkage groups and genetic linkage maps. The markers were sorted in linkage groups with MSTmap (Wu et al., 2008). The linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Previously developed SSR and DArT marker data in the SHA3/CBRD x Naxos population (Lu et al., 2012) were added to the SNP marker data. Genotyped SSR marker data from the Soru#1 x Naxos population was added to the SNP marker data of this population.

For both populations, markers belonging to linkage groups assigned to the same chromosomes based on the BLASTn search were loaded into Join Map v. 4.0 (Van Ooijen, 2006) and the linkage groups were refined using the maximum likelihood mapping algorithm. The genetic distances between markers were calculated by converting recombination fractions into map distances (cM) based on the Kosambi mapping function with minimum LOD score of 3.0 (Kosambi, 1943). To develop maps with many shared markers between the two populations, shared markers between the two populations were found and the function "fixed order" was utilized in JoinMap. Some of these common markers were removed from the maps during the refining, but many remained.

QTL mapping

Interval mapping (IM) and multiple QTL mapping (MQM) was performed on both populations using the software MapQTL6 (van Ooijen, 2011) to detect QTL. Interval mapping (IM) was conducted to detect possible major QTL for powdery mildew resistance. The LOD profiles from interval mapping were observed, and markers closely linked to each QTL that showed effects in several environments were used as cofactors in multiple QTL mapping (MQM). The LOD significance threshold level of powdery mildew was set to 3.2 for SHA3/CBRD x Naxos and 3.4 for Soru#1 x Naxos after a permutation test with 1000 permutations, and was used for declaration of a QTL. QTL reaching this level in one environment either in IM or MQM were also reported for other environments even though their LOD

scores were under the threshold. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by each QTL. Genetic maps and LOD curves were drawn in the software MapChart, v.2.1 (Voorrips, 2002).

Results

Phenotypic analysis

Powdery mildew severity histograms show continuous distributions with transgressive segregation in all testing environments. The 2012 season in Vollebekk experienced less favourable conditions for powdery mildew development than the 2013 and 2016 seasons. Maximum powdery mildew severity from Soru#1 x Naxos RILs in Vollebekk 2012 was approximately 60%, while it exceeded 75% in all other environments. Histograms of mean powdery mildew severity of the Soru#1 x Naxos RILs from Staur 2013 and Vollebekk in 2012, 2013 and 2016 are shown in Fig. S1.

Correlation and heritability

In Soru#1 x Naxos, the powdery mildew severity for the years 2012, 2013 and 2016 in both locations were all significantly ($p < 0.001$) correlated. Days to heading (DH) showed a weak negative correlation with PM in all testing environments, but the relationship was significant only for Vollebekk 2013. The PM heritability (h^2) calculated from the ANOVA table was high for all testing environments with h^2 estimates 0.68 for Vollebekk 2012, 0.86 for Vollebekk 2013, 0.85 for Vollebekk 2016 and 0.84 for Staur 2013 (Table S1)

QTL analysis: SHA3/CBRD x Naxos

Of the 81 587 SNP Chip from Illumina, 9 230 SNP markers were scored as polymorphic in the SHA3/CBRD x Naxos population using the Genome Studio software. The SNP markers were sorted into 45 linkage groups and based on

BLASTn searches the linkage groups were assigned to chromosomes. In addition, the 566 SSR, DArT and gene-specific markers genotyped by Lu et al. (2012) were added to the marker set. Out of the 9 230 SNP dataset, 3512 SNPs were placed on the map after removing redundant markers, and of the 566 SSR and DArT marker dataset, 224 SSR, DArT and gene-specific markers were placed on the map. The map spanned 3130 cM, covering all 21 chromosomes.

In QTL IM analysis, QTL for powdery mildew resistance were detected on chromosomes and chromosome arms 1A, 1RS, 2A, 2B, 2D and 3D in several environments. SNP markers in close proximity to detected QTL were chosen as cofactors in the MQM analysis. After MQM analysis major QTL were detected on 1AS, 1AL, 2BL, 2DS and 2DL (Table 1). Minor QTL were detected on 1AL, 1RS, 2AL, 5AS and 7DS and two minor QTL on 5AL (Table 2). Of the QTL detected, the QTL on 1AS, 2AL, 2BL, 2DL, 5AS, 7DS and one of the two detected on 1AL and 5AL had resistance from Naxos, while the rest had resistance from SHA3/CBRD (Table 1).

[Table 1]

QTL with resistance from Naxos

A major QTL mapped on 1AS with resistance from Naxos was consistent across all environments except Staur 2009 and 2010. The QTL was flanked by markers *wPt-4811* and *wsnp_JD-c7522_8606553*, and explained 0.5-14.4% of the phenotypic variation (Table 1, Fig. 1a).

On the long arm of chromosome 1A, a new major QTL with resistance from Naxos was detected. This QTL was consistent across all environments except Staur 2009, Nanjing 2009, Beijing 2010 and Anyang 2010. This QTL explained 0.6–10.3% of the phenotypic variation (Table 1). Several SNP markers mapped close to the QTL, the markers *Excalibur_c50394_199* and *wsnp_Ex_c22284_31478675* flanking one side of the QTL and the marker *cfa2129b* flanking the other side of the QTL (Fig. 1b).

A new QTL with resistance from Naxos was also detected on 2AL. This QTL had a LOD score above 3.2 in Staur 2008 and in Vollebekk 2008 and 2010. This QTL explained 0-4.7% of the phenotypic variation (Table 1). Only few markers mapped in this QTL region, but the SNP marker *Tdurum_contig13653_471* mapped in the QTL area, and the two DArT markers *tPt_8937* and *wPt_3114* mapped 1-1.5 cM away from the QTL peak (Fig. S2a; Fig. 3).

As in the study by Lu et al. (2012), a major QTL was discovered on chromosome 2BL. The QTL was consistent across all environments except Staur 2008, Vollebekk 2010 and Anyang 2010 in the present study. This QTL explained 2.9 -13.4% of the phenotypic variation (Table 1). The QTL was difficult to map precisely, it consisted of several linked QTL with different LOD scores over the testing environments. In the overall mean data of the QTL, the SNP markers *Excalibur_rep_c67411_210*, *Excalibur_c69493_1208* and *Tdurum_contig42095_3235* mapped closest to the QTL peak with the highest LOD score.

Two new QTL with resistance from Naxos were mapped on chromosome 5A, one on the short arm of the chromosome and one on the long arm. The 5AS QTL had a LOD score above 3.2 in the overall mean data. This QTL explained 0.3-2.8% of the phenotypic variation (Table 1). The QTL had several SNP markers surrounding it, of which *Excalibur_c4964_275* and *BS00067096_51* mapped closest to the QTL (Fig. S2b). The QTL on 5AL had a LOD score above 3.2 at Staur in 2008. This QTL explained 0.0– 4.8% of the phenotypic variation (Table 1). The two SNP markers *BS00021669_51* and *BS0002215_51* mapped closest to the peak of the QTL (Fig S2c).

The two QTL previously detected by Lu et al. (2012) on 2DL and 7DS were also detected in the study. No additional markers were mapped in the area of the QTL, only SSR and DArT markers previously mapped by Lu et al. (2012). The 2DL QTL explained 3.6-17.3% of the phenotypic variation and had a LOD score above 3.2 in all testing environments except Vollebekk 2008, Nanjing 2009 and Anyang 2010 (Table 2). As in the study by Lu et al. (2012) the SSR markers *gwm320* and *mag3616* mapped in the QTL area (Fig. 1d). The 7DS QTL was significant only in Nanjing 2009, and it explained 9.7% of the phenotypic variation this year and testing environment (Table 1). The SSR marker *wmc438* mapped in the QTL area as in the study by Lu et al. (2012) (Fig. S2d).

[Fig. 1]

QTL with resistance from SHA3/CBRD

QTL originating from SHA3/CBRD were detected on four chromosomes.

A QTL on chromosome 1AL with resistance from SHA3/CBRD had a LOD score above 3.2 in Norway, at Vollebekk and Staur in 2008 and 2010. This QTL explained from 0.1-7.1% of the phenotypic variance (Table 1; Fig. S3a).

A second QTL derived from SHA3/CBRD was detected on 1RS. This QTL had a LOD score above 3.2 in Vollebekk 2009 and 2010 and explained 1.2-6.5% of the phenotypic variation (Table 1; Fig S3b).

The major QTL on 2DS with SHA3/CBRD as the resistance source was like the QTL on 1AL only significant in Norway, with a LOD score above 3.2 at Vollebekk in 2008 and 2010 and Staur in 2010. This QTL explained 0.1-12.6% of the phenotypic variation (Table 1; Fig. S3c).

A fourth QTL detected with resistance from SHA3/CBRD was located on 5AL. This QTL was highly significant in Staur 2009 with an explained phenotypic variation of 16.3%, but had a LOD score below 3.2 in all other testing environments (Table 1; Fig. S3d).

QTL analysis: Soru#1 x Naxos

Of the 81 587 SNP SNPs on the Chip from Illumina, 10 500 SNPs were polymorphic in the Soru#1 x Naxos population. By MST mapping these markers were assembled into 83 linkage groups assigned to chromosomes based on a BLASTn search. In addition to the SNP markers, 50 SSR markers were added to the dataset. Of the 10 500 polymorphic SNPs, 4113 were discarded due to poor quality. In total 6387 SNP markers and 50 SSR markers were loaded into JoinMap v. 4.0 for map construction. Out of these markers, 2788 SNP markers and 36 SSR markers were included in maps spanning 3031 cM, covering all chromosomes.

In QTL Interval Mapping (IM), QTL for PM resistance were detected on chromosome 1AS, 2AL, 2BL and 3AS in most environments. Markers in close proximity to the detected QTL in IM were chosen as cofactors in Multiple-QTL model (MQM) mapping. After final MQM mapping, four major QTL were identified on chromosomes 1AS, 2AL, 2BL and 3AS. The major QTL on 1AS, 2AL and 2BL had resistance from Naxos, the major QTL on 3AS was contributed by Soru#1 (Table 2).

[Table 2]

Major QTL with resistance from Naxos

The major QTL detected on chromosome 1AS was consistent across all environments. It explained 9.8-18.7% of the phenotypic variation (Table 2). There were not many markers mapped to the QTL region of this chromosome, but

three SNP markers mapped in close proximity to the QTL; *Excalibur_c105151_200*, *Kukri_c11891_1015* and *Tdurum_contig50845_25*. This QTL mapped in the same area as the 1AS QTL in SHA3/CBRD x Naxos population. The SNP markers *BS00022701_51*, *Kukri_c11891_1015*, *Kukri_rep_c81545_195* and *wsnp_Ex_c64327_63176640*, and SSR markers *cnl137*, *gwm33b* and *gwm33a* mapped in the QTL area in both populations, verifying that the 1AS QTL is the same (Fig. 2).

[Fig. 2]

The major QTL on 2AL had a LOD score of 3.07 at Staur 2013 and a LOD score above 3.4 at Vollebekk 2013 and 2016. This QTL explained from 6.1-9.7% of the phenotypic variation (Table 2). The two SNP markers *Bobwhite_c6356_87* and *Tdurum_contig13653_4712* mapped in close proximity of the QTL on this chromosome. The QTL on 2AL in Soru#1 x Naxos mapped in the same region as the 2AL QTL in SHA3/CBRD x Naxos. This QTL on 2AL could be verified by the SNP markers *Ex_c28017_641*, *Td_con13653_471* and *Bwc17403_635*, which mapped in the same area around the QTL both in Soru#1 x Naxos and SHA3/CBRD x Naxos (Fig. 3).

[Fig. 3]

A third major QTL with resistance from Naxos was located on 2BL and had a LOD score above 3.4 at Staur and Vollebekk in 2013. This QTL explained 6.9-15.8% of the phenotypic variation (Table 2). In this part of the 2BL chromosome many markers were mapped, giving a narrow QTL peak with several flanking SNP markers. The two SNP markers most closely linked to the QTL were *wExrc73919_71799491* and *wExc51661_55531646* (Fig.S4). The QTL on 2BL in Soru#1 x Naxos was mapped approximately 30 cM away from the QTL on 2BL in the SHA3/CBRD x Naxos population, and had different markers in the QTL area.

Major QTL with resistance from Soru#1

The last major QTL detected in the Soru#1 x Naxos was located on the short arm of chromosome 3A and had resistance from Soru#1. This QTL had a LOD score above 3.4 at Vollebekk in 2012, and LOD scores above 2.6 the other years and explained 5.9-7.8% of the phenotypic variation (Table 2; Fig S5).

Discussion

Naxos has previously been demonstrated to be a good source for partial resistance to powdery mildew by Lillemo et al. (2010) and Lu et al. (2012). With the use of the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos, and development of high-density marker maps with both SNP, SSR, DArT and gene-specific markers, we have been able to detect more QTL with resistance from Naxos, and in addition validate QTL on chromosome arms 1AS and 2BL previously detected by Lu et al. (2012).

Phenotypic evaluation Soru#1 x Naxos:

For the Soru#1 x Naxos population, powdery mildew severity histograms show continuous distributions with transgressive segregation in all testing environments indicating that both parents carry resistance genes to powdery mildew. This was further demonstrated in the QTL analysis, where QTL for powdery mildew resistance came from both parents.

Correlation and heritability:

Correlation between days to heading (DH) and powdery mildew severity was negative in all testing environments. Lines heading early are exposed to powdery mildew infection over a longer time period compared to lines heading later, resulting in a higher infection rate in these "earlier" lines and a negative correlation with the DH. The heritability estimates for the Soru#1 x Naxos population for powdery mildew were high in all environments, indicating that genetic factors play an important role in this disease.

High-density molecular marker maps with SSR, DArT and SNP markers:

Previous reports using SSR, DArT and RFLP markers have conducted mapping with a few hundred polymorphic markers placed on map (Asad et al., 2014; Lu et al., 2012; Somers et al., 2004). The 90K SNP Chip from Illumina has enabled the development of marker maps with a higher number of markers, increasing the probability of detecting,

and linking a marker to a QTL of interest (Wang et al., 2014). Development of mapmaking programs alongside with the development of high-density SNP chips also make it feasible to construct integrated maps with different marker-types, giving an even better map resolution and making it possible to exchange previously used SSR, DArT and RFLP markers with SNP markers. The SNP markers are easy to utilise in genotyping with today's technology platforms, e.g. KASP (Semagn et al., 2014).

The QTL mapping study in SHA3/CBRD x Naxos by Lu et al. (2012) revealed QTL for powdery mildew resistance on chromosome arms 1AS, 1RS, 2BL, 2DLc, 2DL, 6BL, 7AL and 7DS, where the QTL on 1AS, 2BL, 2DL and 7DS originated from the resistance source Naxos. In the present study, with the use of the 90K SNP chip in addition to the previously used SSR, DArT and gene-specific markers, additional QTL were detected on 1AL, 2AL, 5AS and 5AL with Naxos as resistance source and from SHA3/CBRD additional QTL on 1AL and 3BS were detected. The QTL on 1AS and 2BL with Naxos as resistance source detected by Lu et al. (2012) were validated in this study. The QTL on 2DL and 7DS detected in the study were the same QTL as detected by Lu et al. (2012), but no SNP markers mapped close to the QTL on these two chromosomes in the present study.

Comparison with previous reports:

Chromosome arm 1AS harbours the major resistance gene *Pm3*, encoding seven alleles conferring resistance to different races of *Blumera graminis* f.sp. *tritici* (Tommasini et al., 2006). The resistance on 1AS from Naxos was suggested to be race-non-specific by Lu et al. (2012) where it was demonstrated that Naxos lacks the *Pm3* gene based on *UP3B/UP1A* and *Pm3MF/Pm3ER1* primers. In the present study the 1AS QTL was more precisely mapped with the addition of SNP markers to the map. Moreover, it was also validated in the Soru#1xNaxos population where it was significant in both years and testing locations. The QTL explained a high proportion of the phenotypic variance in both populations, pointing to the 1AS QTL as an important APR QTL for PM resistance.

Two QTL were detected on chromosome arm 1AL in SHA3/CBRD x Naxos, one originating from Naxos. Several other studies have detected QTL for race-non-specific resistance in the centromeric and *cfa2129* marker area of 1AL.This includes QTL detected in winter wheat line RE714 (Mingeot et al., 2002), the spelt variety Oberkulmer (Keller et al., 1999) and Chines winter wheat Bainong 64 (Lan et al., 2009). Based on mapping data in the above mentioned studies, it is likely that the QTL from Naxos detected in SHA3/CBRD x Naxos is the same QTL, suggesting that there is an APR QTL at this locus effective in several environments and genetic backgrounds.

The 2AL QTL derived from Naxos detected in both SHA3/CBRD x Naxos and Soru#1 x Naxos was flanked by several common markers in the two populations, strongly indicating that this QTL is the same in both populations. Powdery mildew QTL on 2AL has also been detected in the winter wheat cultivar Massey by Liu et al. (2001) and in a derivative of Massey, USG3209, by Tucker et. al (2007). Comparison of the integrated maps in the present study and the mapping data from Liu et al. (2001) and Tucker et. al (2007) suggests the 2AL QTL derived from Naxos to be the same 2AL QTL as detected in Massey and USG3209.

There have been several reports of a QTL for PM resistance on 2BL. Both in the winter wheat cv. Massey ((Liu et al., 2001), the Japanese wheat cultivar Fukuho-komugi (Liang et al. (2006) , in the line RE9001 ((Bougot et al., 2006), in the line USG3209 ,((Tucker et al., 2007) and in the Chinese wheat cultivar Lumai 21 (Lan et al. (2010). Lan et al. (2010) suggested this 2BL QTL in Massey, Fukuho-komugi, RE9001, USG3209 and Lumai 21 to be at the same or closely linked loci according to their position and flanking markers in the wheat SSR consensus map (Somers et al., 2004). In the present study, a QTL on 2BL from Naxos was detected in both SHA3/CBRD x Naxos and Soru#1 x Naxos. In the SHA3/CBRD x Naxos population this 2BL QTL was verified to be the same as the QTL detected by Lu et al. (2012) in Naxos due to the common markers flanking the QTL in both studies. In addition, based on mapping data and detection of common markers, the QTL from Naxos in SHA3/CBRD x Naxos is possibly also the same as the QTL detected in Massey, Fukuho-komugi, RE9001, USG3209 and Lumai 21. A 2BL QTL with resistance from Naxos was also detected in the Soru#1 x Naxos population in the present study. When investigating the mapping data for both populations it is likely that this 2BL QTL is not the same as the QTL detected with resistance from Naxos in the SHA3/CBRD x Naxos population. The QTL on 2BL detected in Soru#1x Naxos is possibly a newly discovered QTL, but this needs further investigation.

A 5AS QTL significant in Nanjing 2009 was detected in the present study. In the area of the detected QTL several minor QTL were also detected, these might be linked to the major QTL. A 5AS QTL for APR have also been reported in *Triticum militinae* ((Jakobson et al., 2006), and in the Swiss spelt variety Oberkulmer (Keller et al. (1999). The mapping data suggests the QTL detected in the studies by Keller et al. (1999) and Jakobson et al. (2006) to be the same QTL and in addition to be closely linked to the QTL detected in SHA3/CBRD x Naxos with resistance from Naxos in our study.

The major QTL detected on 5AL with resistance from Naxos was significant at Staur in 2008. There have been several reports of QTL for APR to PM on 5AL; the CIMMYT bread wheat line Saar (Lillemo et al., 2008), the Swedish winter wheat cultivar Folke (Lillemo et al., 2012), the Swiss spelt variety Oberkulmer (Keller et al., 1999) and the the DH line 8.1.8.1 x T, made from a cross of the spring wheat cv. Tahti with tetraploid *T. militinae, (Jakobson et al., 2012).* , Studies of the maps and markers in these different lines and cultivars and comparisons with the wheat composite map (graingenes.com; http://wheat.pw.usda.gov/GG3/) suggests the 5AL QTL found in Saar, Folke, Oberkulmer, 8.1.8.1 x T and Naxos to be the same or closely linked QTL, but this needs further study.

In conclusion, high density marker maps with SSR, DArT and SNP markers were developed in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. With these new high-density maps twelve QTL were detected in the SHA3/CBRD x Naxos population, eight of them with Naxos as the resistance source. Of these, the QTL on 1AS and 2BL are verified as the same QTL as detected by Lu et al. (2012). These two QTL are now more precisely mapped, and the identification of closely linked SNP markers will greatly facilitate the use of these QTL for resistance breeding. The QTL on 1AS was in addition further confirmed in the Soru#1 x Naxos population. The new QTL detected in SHA3/CBRD x Naxos on chromosome 2AL, possibly the same QTL that has previously been reported in Massey and USG3209, was also verified in the Soru#1 x Naxos population, making also this QTL highly interesting for resistance breeding.

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Fig. 1: Segments of chromosomes with resistance QTL derived from Naxos. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosomes. A threshold of 3.2 is indicated as a dashed line in the LOD graphs. DArT and SSR markers also mapped in the study by Lu et al. (2012) marked in blue **a)** Chromosome 1AS **b)** Chromosome 1AL, **c)** Chromosome 2BL **d)** Chromosome 2DL.

Fig. 2 Segment of chromosome 1AS with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome in SHA3/CBRD x Naxos and right of the chromosome in Soru#1 x Naxos. A threshold of 3.2 is indicated as a dashed line in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a dashed line in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in green, and markers also mapped in the QTL area by Lu et al., (2012) marked in blue. Dotted lines show the position of the common markers between the two populations.

Fig. 3 Segment of chromosome 2AL with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.2 is indicated as a dashed line in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a dashed line in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in green, dotted lines show the position of the common markers between the two populations.

Table 1 Results QTL mapping in the RIL population SHA3/CBRD x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance.

Table 2 Results from QTL mapping in the RIL population Soru#1 x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance.

Fig. S1 Frequency distribution of PM severity in the Soru#1 x Naxos population across the two testing environments Staur 2013 and Vollebekk 2012, 2013 and 2016, together with parental lines

Fig. S2 Segment of chromosome arms with minor QTL with resistance from Naxos. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.2 is indicated as a dashed line in the LOD graph. **a)** Chromosme arm 2AL **b)** Chromosome arm 5AS **c)** Chromosome arm 5AL **d)** Chromosome arm 7DS

Fig. S3 Segment of chromosome arms with QTL with resistance from SHA3/CBRD. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.2 is indicated as a dashed line in the LOD graph

a) Chromosome arm 1AL **b)** Chromosome arm 1RS **c)** Chromosome arm 2DS **d)** Chromosome arm 5AL

Fig. S4 Segment of chromosome 2BL with major QTL with resistance from Naxos. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.4 is indicated as a dashed line in the LOD graph.

Fig. S5 Segment of chromosome arm 3AS with major QTL with resistance from Soru#1. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.4 is indicated as a dashed line in the LOD graph.

Table S1 Pearson correlation coefficients among powdery mildew severities for individual environments, heading dates, and heritability (h^2) estimated for each environment of the Soru#1 x Naxos RILs

Table 1: Results QTL mapping in the RIL population SHA3/CBRD x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance.

		PM severity Vollebekk			PM severity Staur			PM severity China			Overall mean	Source
QTL	**Markers in close proximity**	**PM08v**	**PM09v**	**PM10v**	**PM08s**	**PM09s**	**PM10s**	**PM09nj**	**PM10bj**	**PM10ay**	**Pmallm**	**Source**
1AS	wJD_c7522_8606553, K_c2121_2345	**13.7**	**11.7**	**4.1**	1.3	4.8	0.5	**14.4**	**6.7**	**12.5**	**8.1**	Naxos
1AL	Exc_50394_199, wEx_c22284_31478675, cfa2129b	**5.8**	**4.1**	**10.3**	**5.7**	3.4	**7.9**	1.4	4.8	0.6	**7.4**	Naxos
1AL	Bw_c6664_644, wJD_c6664_7807201	**5.2**	3.2	**5.5**	**7.1**	0.1	**5.6**	1.2	5.1	0.3	**5.2**	SHA3/CBRD
1RS	K_c24684_134, RFL_con5444_1284	2	**6.5**	**4**	1.1	1.2	2.2	1.6	1.5	1.4	2.8	SHA3/CBRD
2AL	Td_con13653_471	**4.3**	1.2	**4.7**	**4.6**	0	2.5	1	0.3	0.3	**3.2**	Naxos
2BL	R875_c66657_91	**6.5**	**7.9**	1.9	1.8	**10.3**	**7.4**	**7.2**	**7.6**	1.4	**4.7**	Naxos
2BL	Td_con42095_3235, Exc_c7446634	**6.2**	**10.9**	2.9	**5.7**	**12**	**13.4**	**7**	**9.6**	3.1	**8.6**	Naxos
2DS	IAAV8527, wBQ161779D_Ta_2_1	**10.5**	4.5	**7.9**	**8.3**	0.1	**12.6**	2.5	0.7	2.9	**8.9**	SHA3/CBRD
2DL	gwm 320,mag3616	5.9	**9.8**	**15.3**	**16.7**	**13.2**	**17.3**	6.4	**10.6**	3.6	**15.5**	Naxos
5AS	Exc_c4964_275, BS00067096_51	1.9	2.7	1.6	1.1	0.8	2.8	3.1	0.77	0.3	**2.7**	Naxos
5AL	BS00022215_51, BS00021669_51	0.9	0.5	1.7	**4.8**	1.9	2.8	0.8	0.4	0	2.2	Naxos
5AL	R875_c13931_205, IAAV8669	2.4	0	0.4	1.9	**16.3**	0.8	0.4	0.8	0	0.1	SHA3/CBRD
7DS	wmc438	6	7.4	2.7	3.8	6.8	3.5	**9.7**	3.9	2.2	7	Naxos

QTL with LOD score above 3.2 highlighted in bold

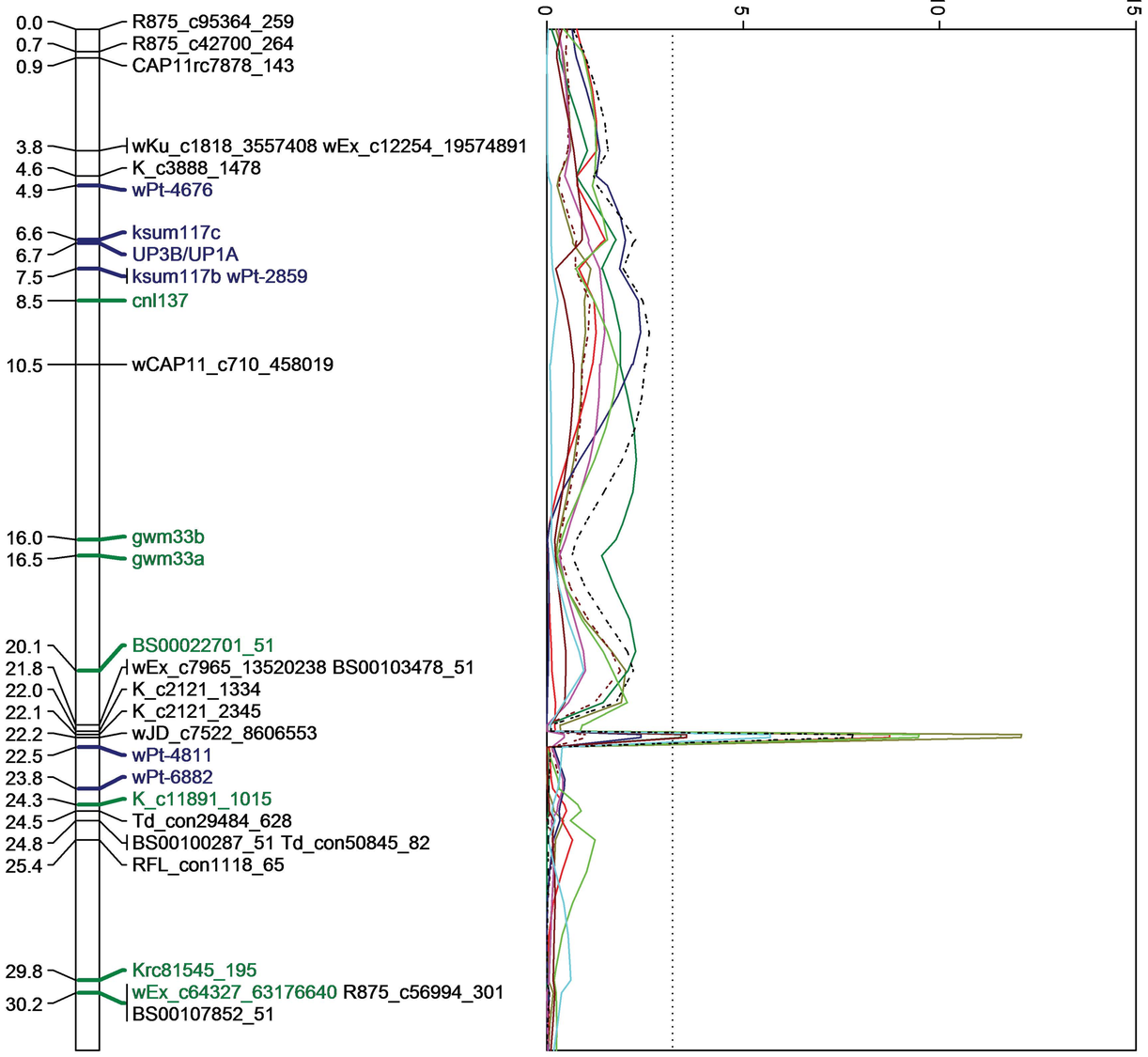
Table 2: Results from QTL mapping in the RIL population Soru#1 x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance.

		PM severity Vollebekk			PM severity Staur	Overall mean	Source
Chrom	**Markers in close proximity**	**PMv12**	**PMv13**	**PMv16**	**PMs13**	**PMallm**	**Source**
1AS	Ex_c105151_200, K_c11891_1015	**18.7**	**11.0**	**20.1**	**9.8**	**17.6**	Naxos
2AL	Bw_c6356_87, Td_c13653_471	6.1	**9.7**	**8.6**	6.8	**8.8**	Naxos
2BL	wExrc73919_71799491, wExc51661_55531646	1.9	**6.9**	3.9	**15.8**	**7.8**	Naxos
3AS	BS00022524_51, wExrc67635_66291944	**7.8**	6.5	5.9	5.9	**8.3**	Soru#1

QTL with LOD score above 3.2 highlighted in bold

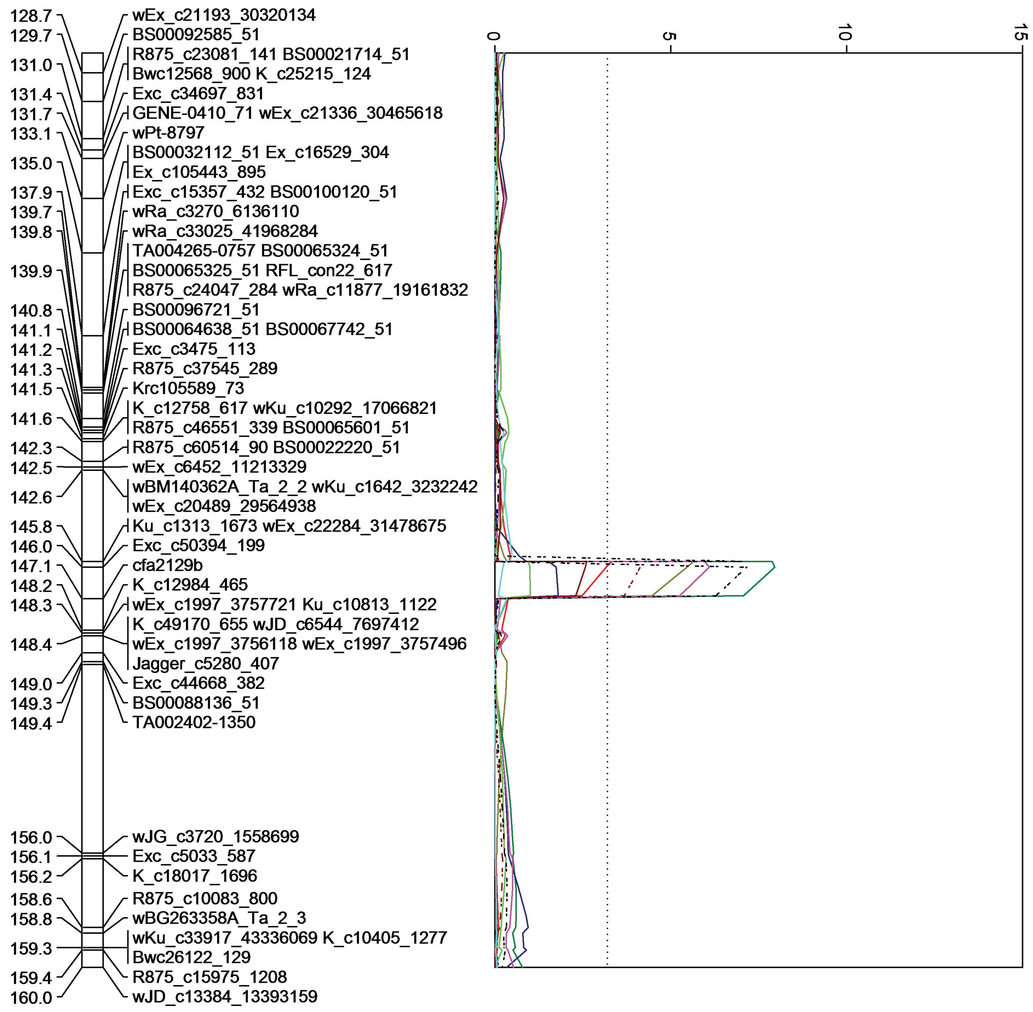
a

SHA3/CBRD x Naxos 1AS



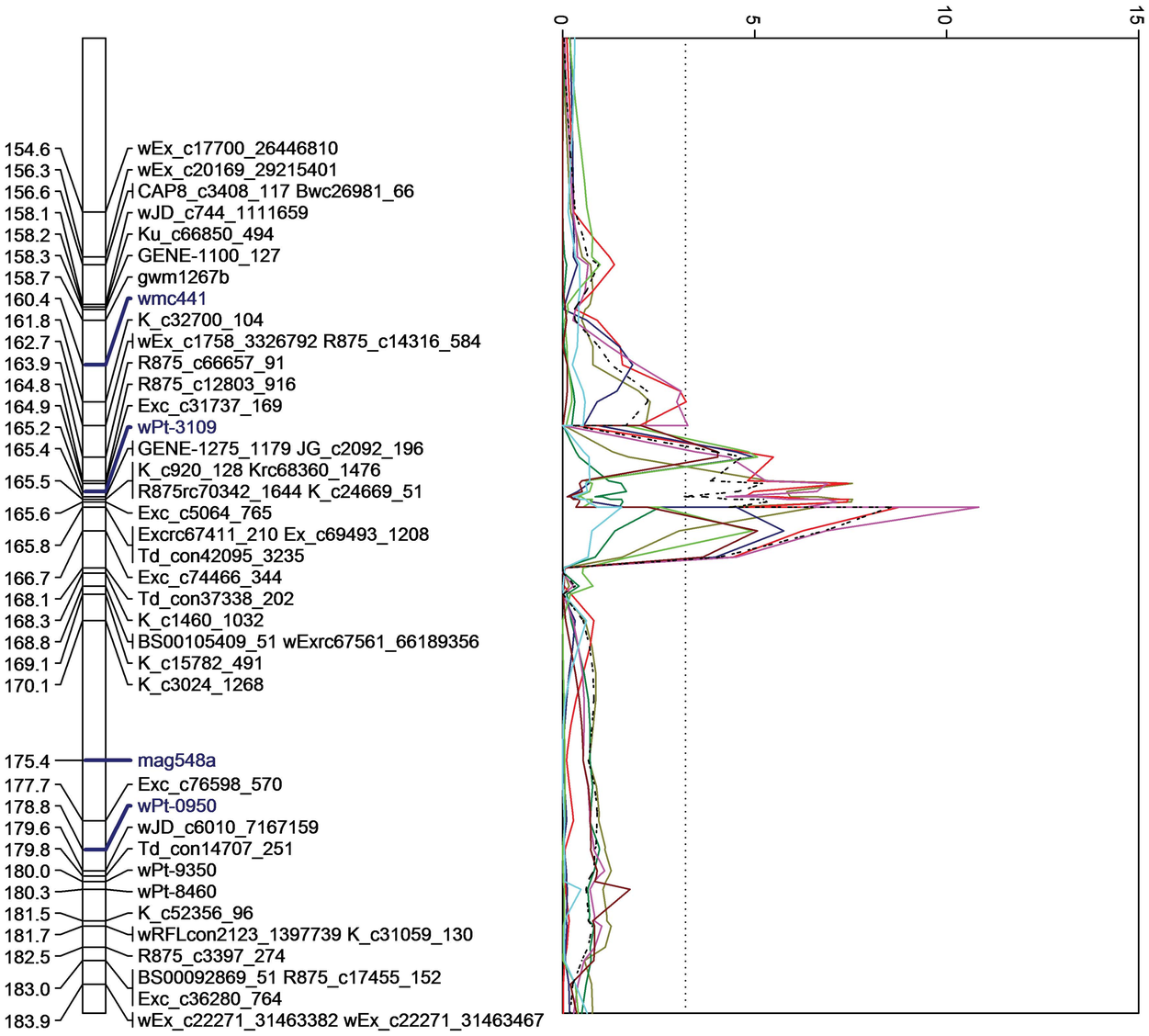
b

SHA3/CBRD x Naxos 1AL



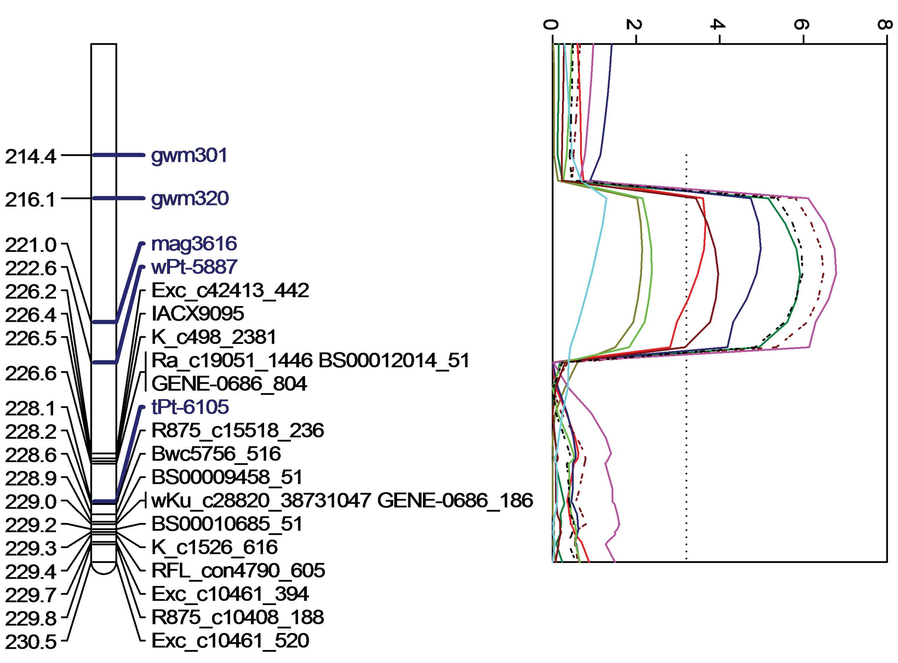
c

SHA3/CBRD x Naxos 2BL



d

SHA3/CBRD x Naxos 2DL



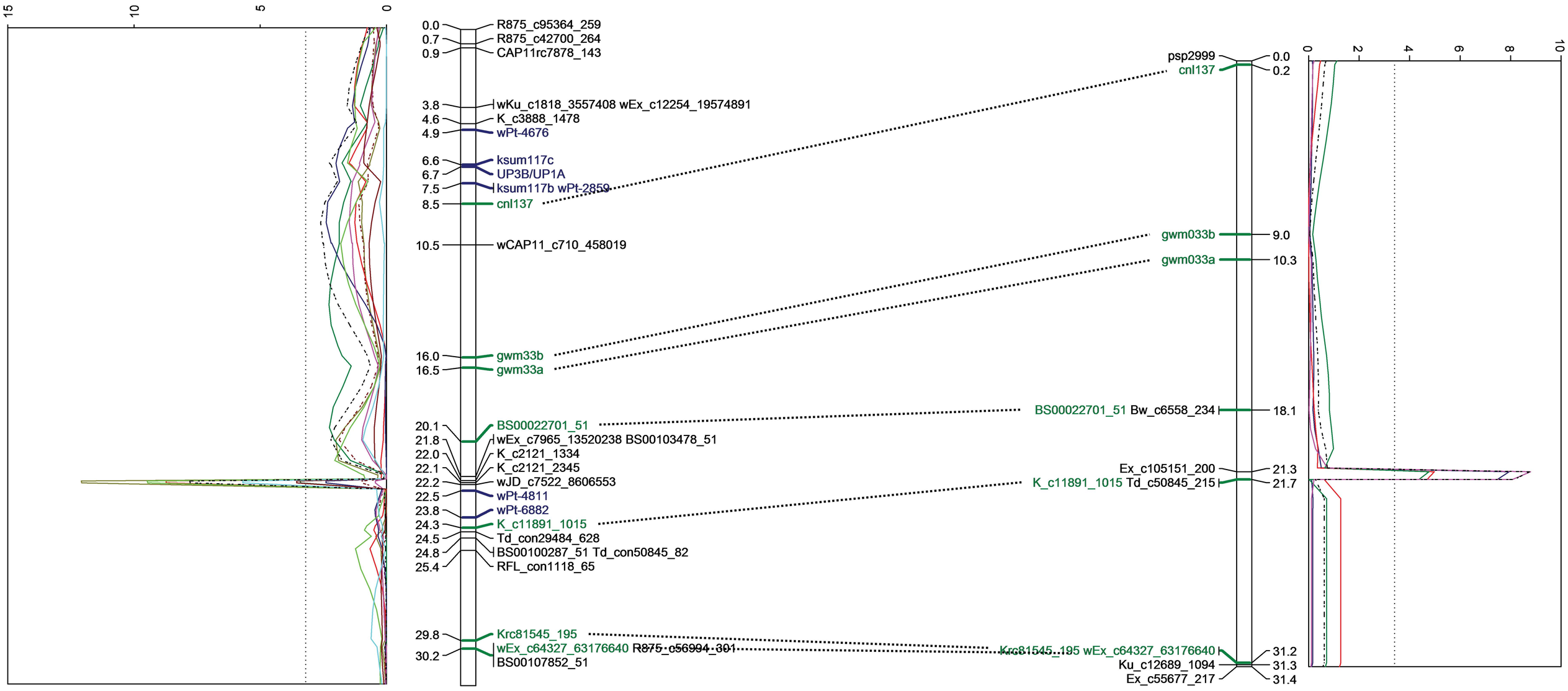
SHA3/CBRD x Naxos

- PM Vollebekk 2008
- PM Vollebekk 2009
- PM Vollebekk 2010
- PM Staur 2008
- PM Staur 2009
- PM Staur 2010
- PM Nanjing 2009
- PM Beijing 2010
- PM Anyang 2010
- PM all over mean

SHA3/CBRD x Naxos

1AS

Soru#1 x Naxos



SHA3/CBRD x Naxos

- PM Vollebekk 2008
- PM Vollebekk 2009
- PM Vollebekk 2010
- PM Staur 2008
- PM Staur 2009
- PM Staur 2010
- PM Nanjing 2009
- PM Beijing 2010
- PM Anyang 2010
- PM all over mean

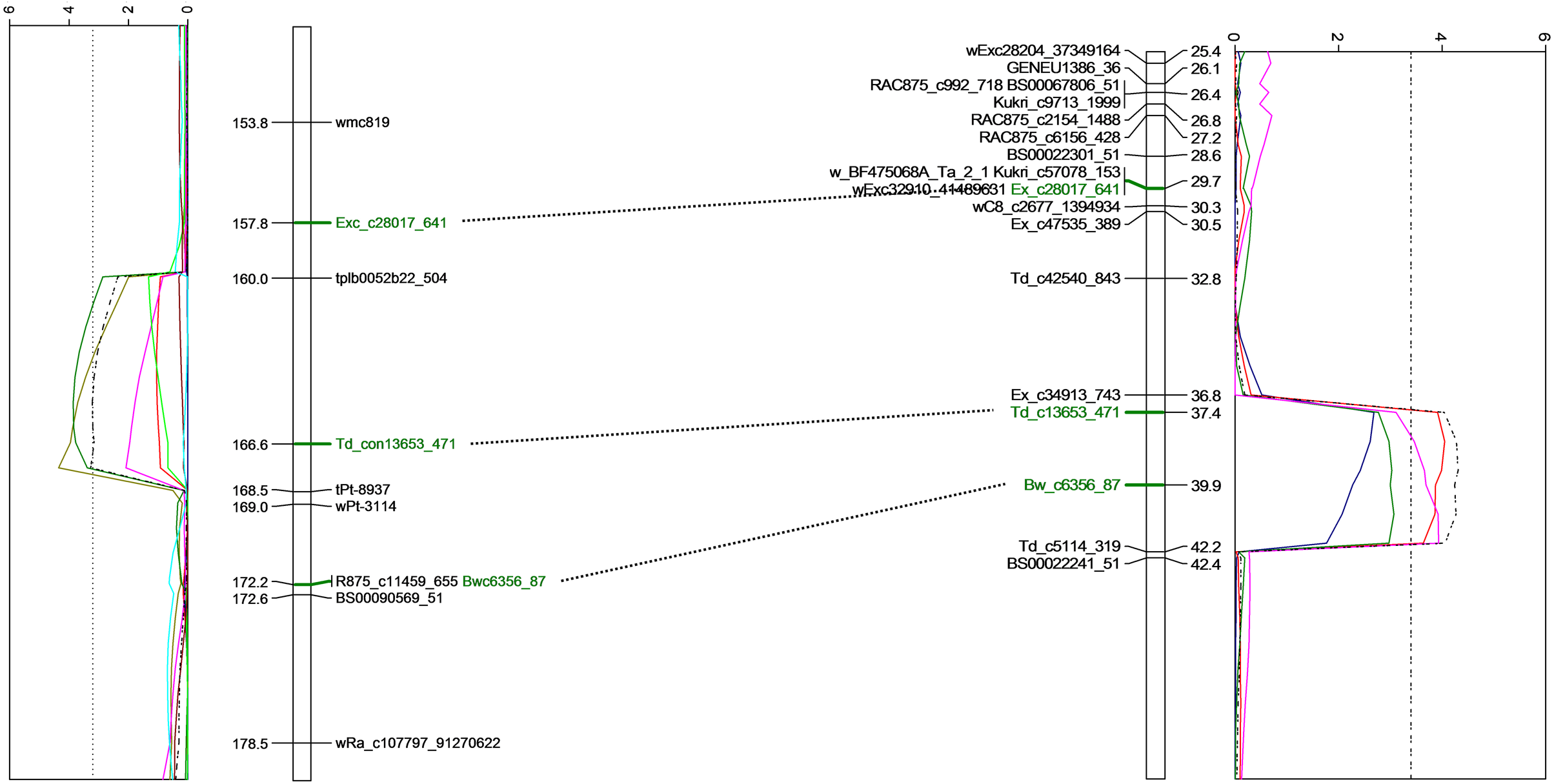
Soru#1 x Naxos

- PM all over mean
- PM Vollebekk 2016
- PM Staur 2013
- PM Vollebekk 2013
- PM Vollebekk 2012

SHA3/CBRD x Naxos

2AL

Soru#1 x Naxos



SHA3/CBRD x Naxos

- PM Vollebekk 2008
- PM Vollebekk 2009
- PM Vollebekk 2010
- PM Staur 2008
- PM Staur 2009
- PM Staur 2010
- PM Nanjing 2009
- PM Beijing 2010
- PM Anyang 2010
- PM all over mean

Soru#1 x Naxos

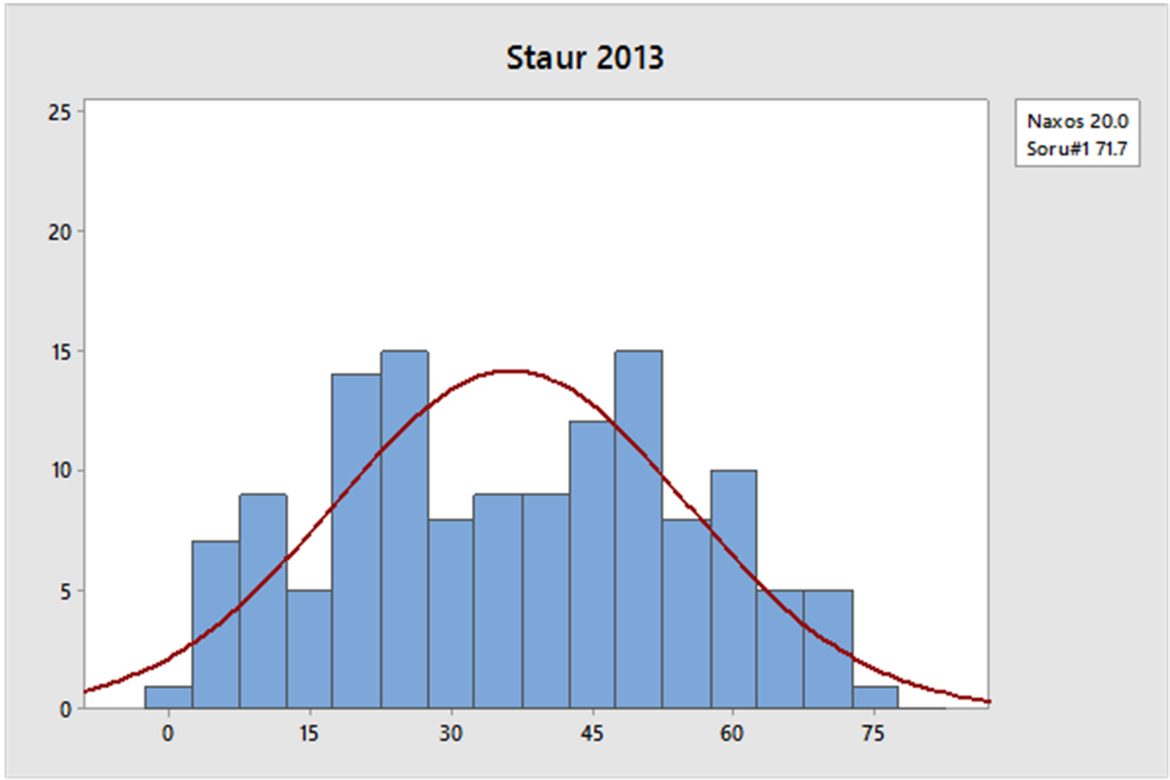
- PM all over mean
- PM Vollebekk 2016
- PM Staur 2013
- PM Vollebekk 2013
- PM Vollebekk 2012

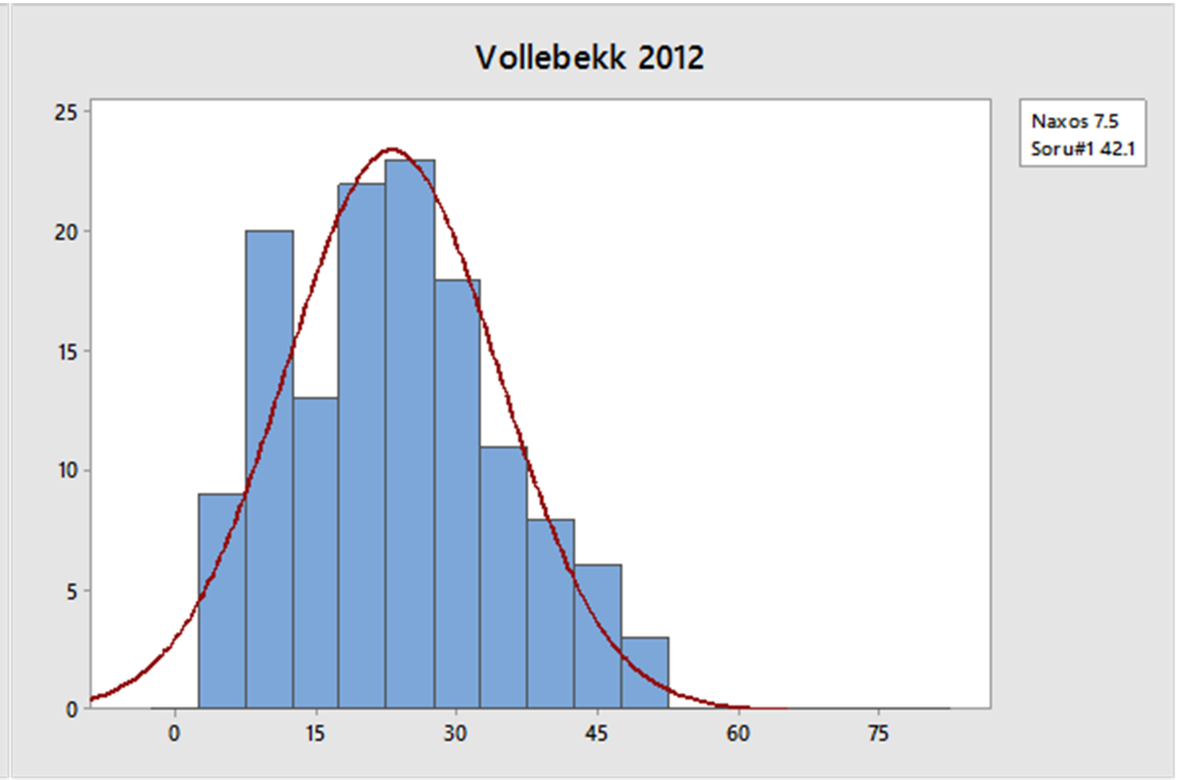
Table S1: Pearson correlation coefficients among powdery mildew severities for individual environments, heading dates, and heritability (h^2) estimated for each environment of the Soru#1 x Naxos RILs

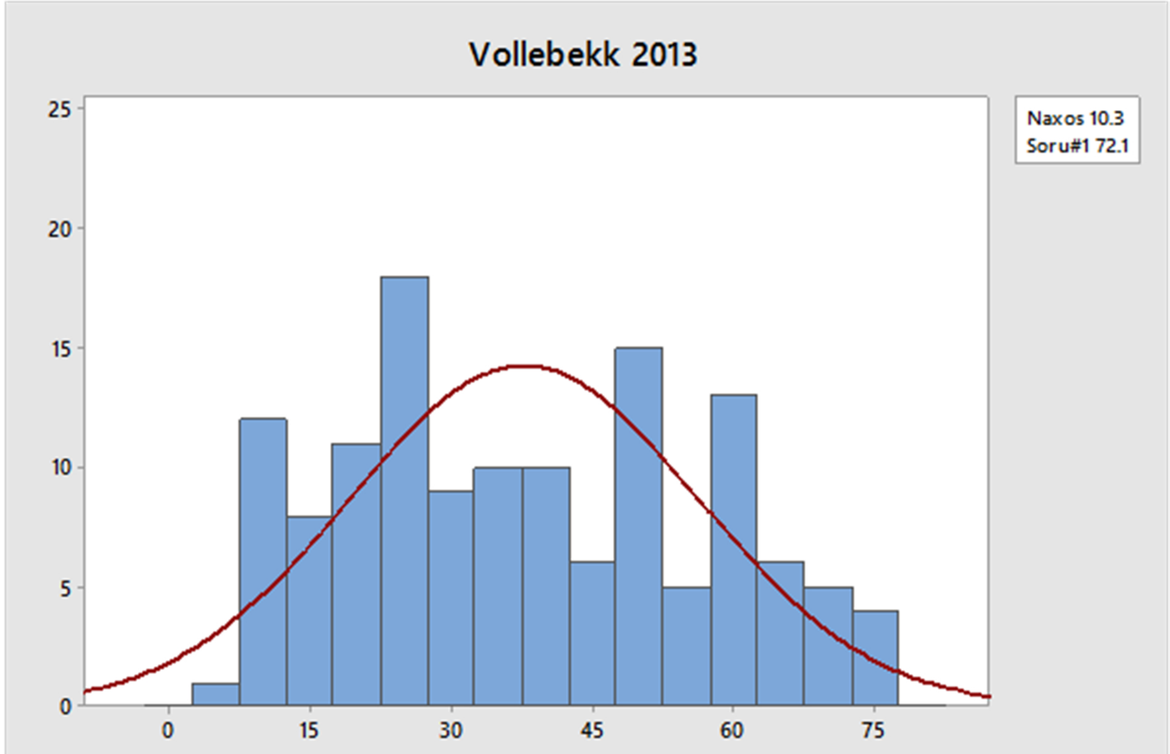
	Vollebekk 2012	**Vollebekk 2013**	**Vollebekk 2016**	**Staur 2013**
Vollebekk 2013	0.777 **			
Vollebekk 2016	0.807 **	0.789 **		
Staur 2013	0.693 **	0.796 **	0.731 **	
DH Staur 2013	-0.125	-0.153	-0.202	-0.071
DH Vollebekk 2016	-0.162	-0.239*	-0.155	-0.201
Heritability (h^2)	0.678	0.858	0.852	0.841

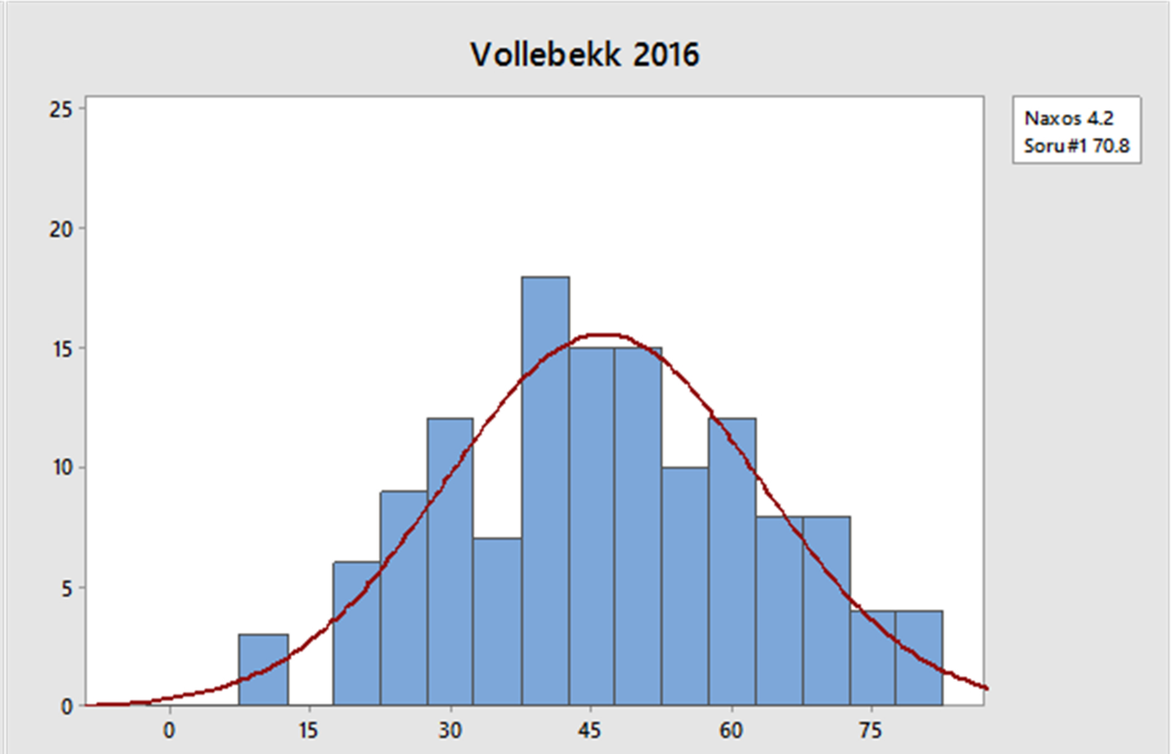
** 0.0001 level, *0.01 level

Line frequency



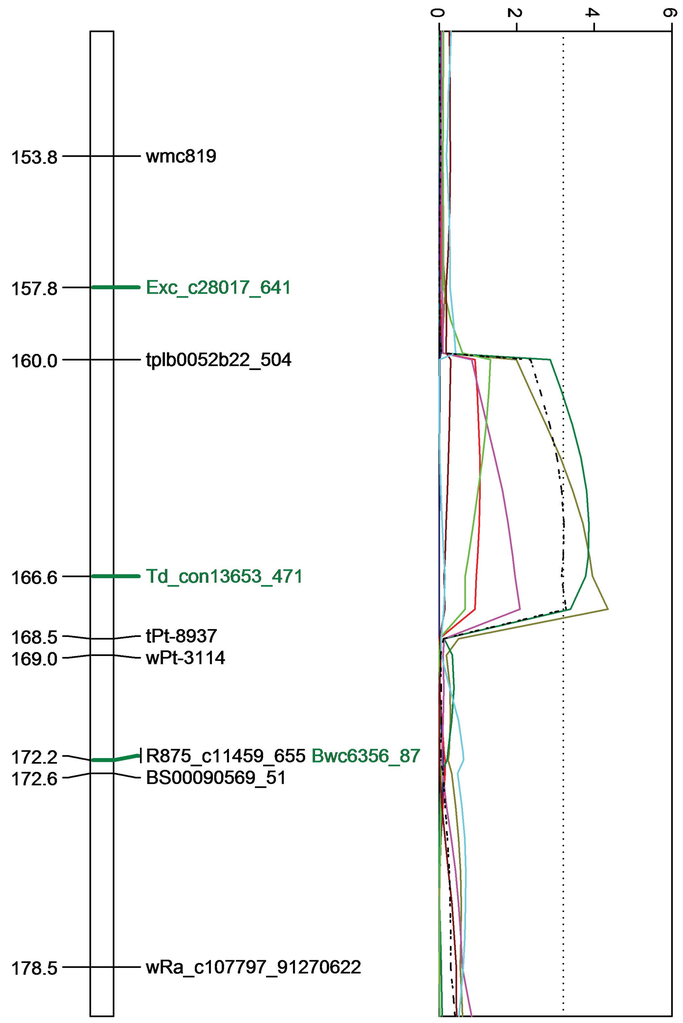




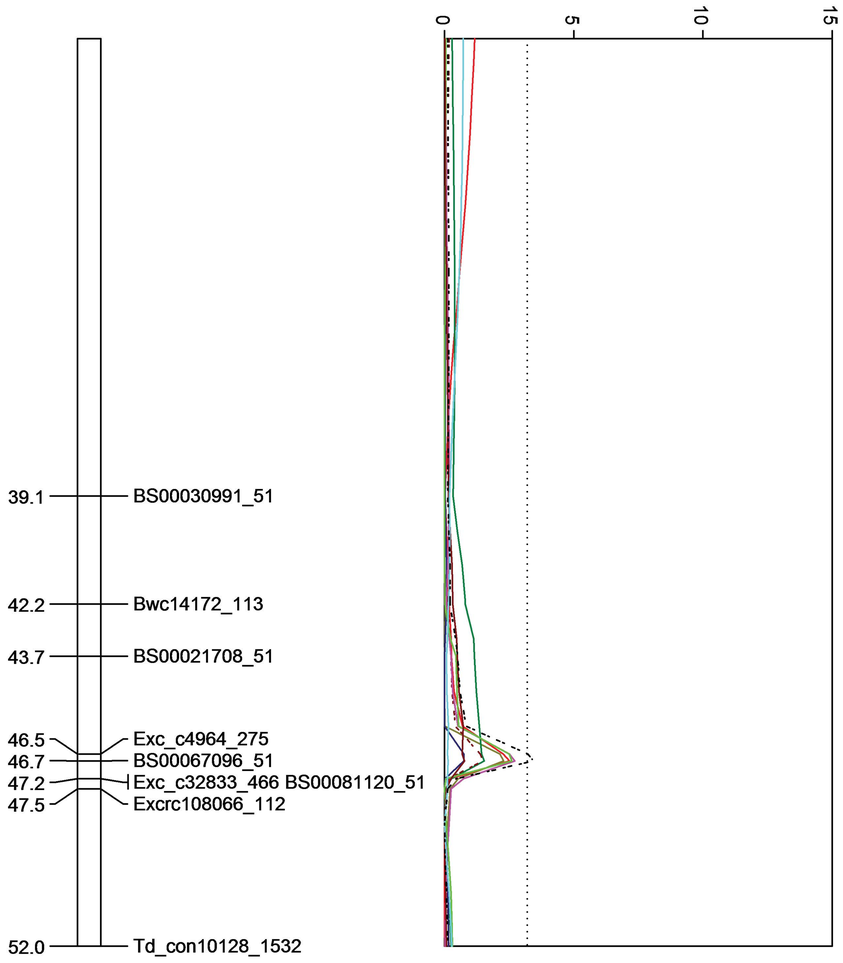


PM severity (%)

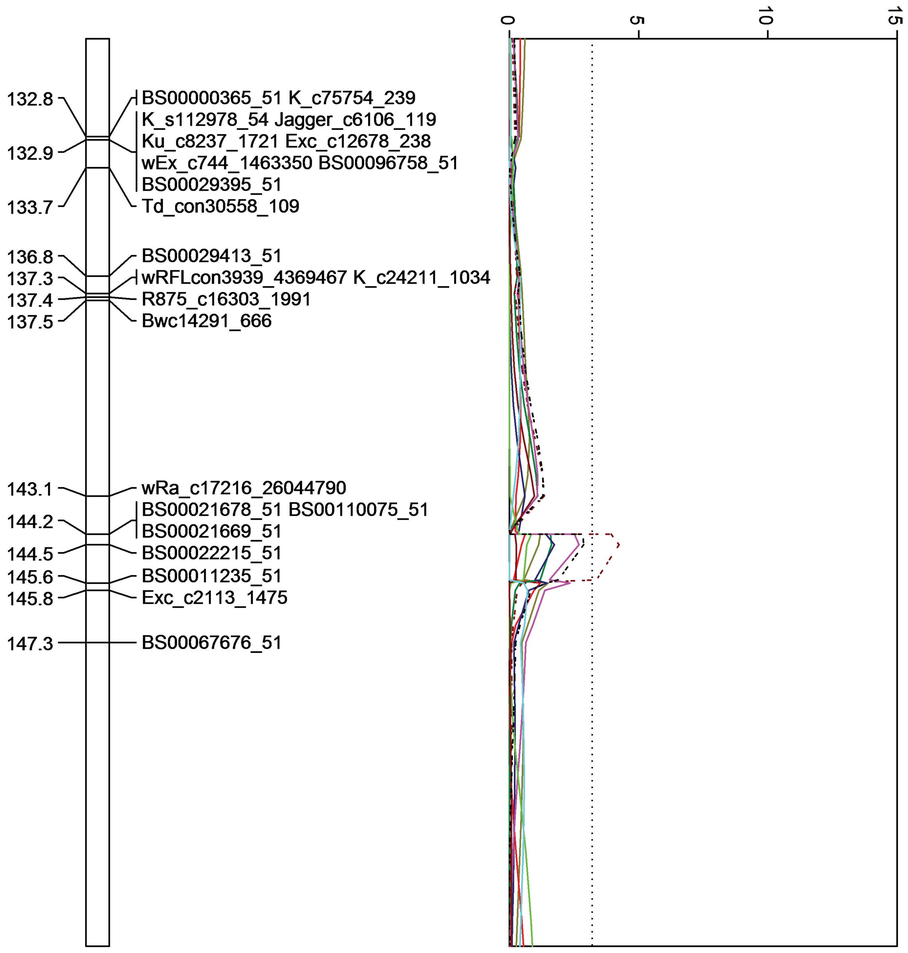
a


SHA3/CBRD x Naxos 2AL
0
2
4
6
153.8 wmc819
157.8 Exc_c28017_641
160.0 tplb0052b22_504
166.6 Td_con13653_471
168.5 tPt-8937
169.0 wPt-3114
172.2 R875_c11459_655 Bwc6356_87
172.6 BS00090569_51
178.5 wRa_c107797_91270622

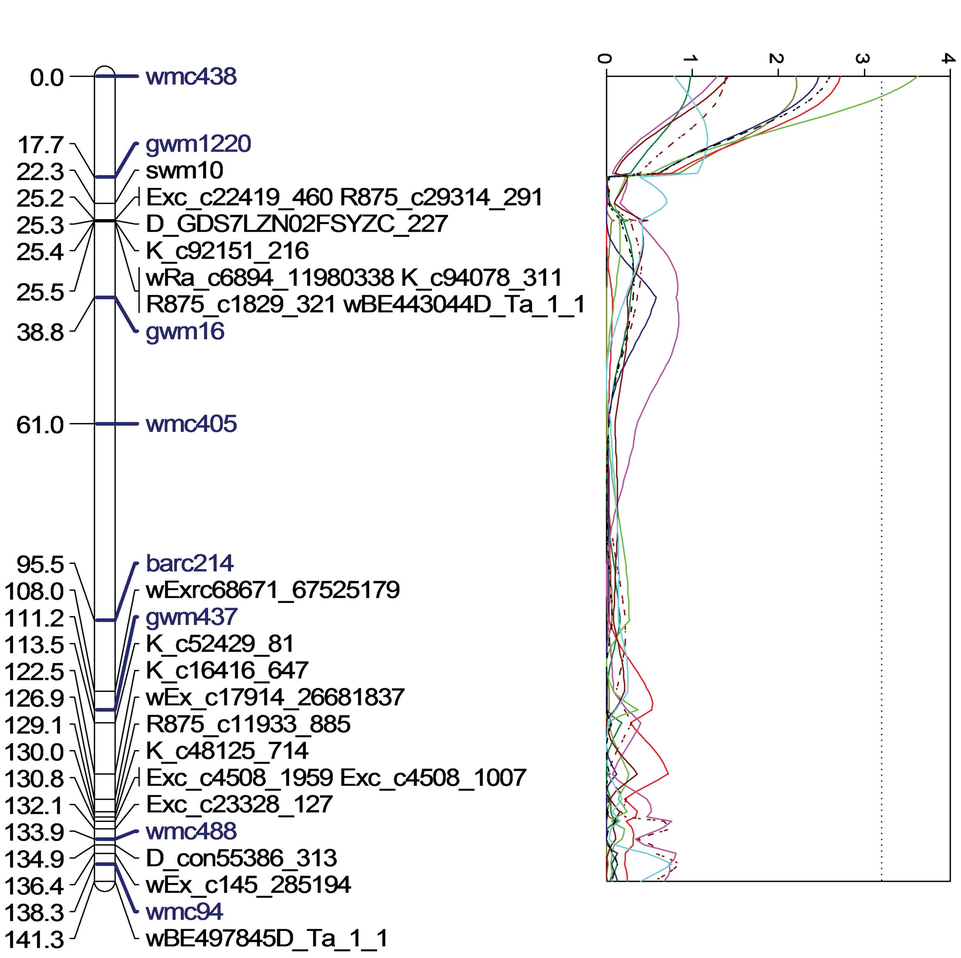
b

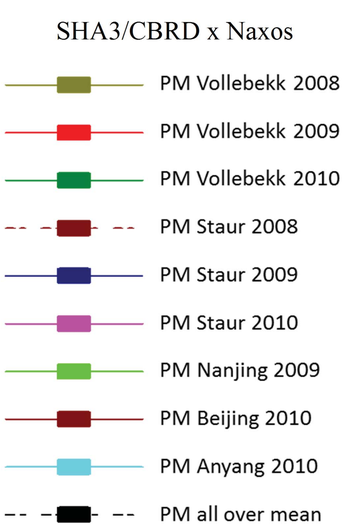

SHA3/CBRD x Naxos 5AS
0
5
10
15
39.1 BS00030991_51
42.2 Bwc14172_113
43.7 BS00021708_51
46.5 Exc_c4964_275
46.7 BS00067096_51
47.2 Exc_c32833_466 BS00081120_51
47.5 Excrc108066_112
52.0 Td_con10128_1532

c


SHA3/CBRD x Naxos 5AL
0
5
10
15
132.8 BS00000365_51 K_c75754_239
K_s112978_54 Jagger_c6106_119
132.9 Ku_c8237_1721 Exc_c12678_238
wEx_c744_1463350 BS00096758_51
133.7 BS00029395_51
Td_con30558_109
136.8 BS00029413_51
137.3 wRFLcon3939_4369467 K_c24211_1034
137.4 R875_c16303_1991
137.5 Bwc14291_666
143.1 wRa_c17216_26044790
144.2 BS00021678_51 BS00110075_51
BS00021669_51
144.5 BS00022215_51
145.6 BS00011235_51
145.8 Exc_c2113_1475
147.3 BS00067676_51

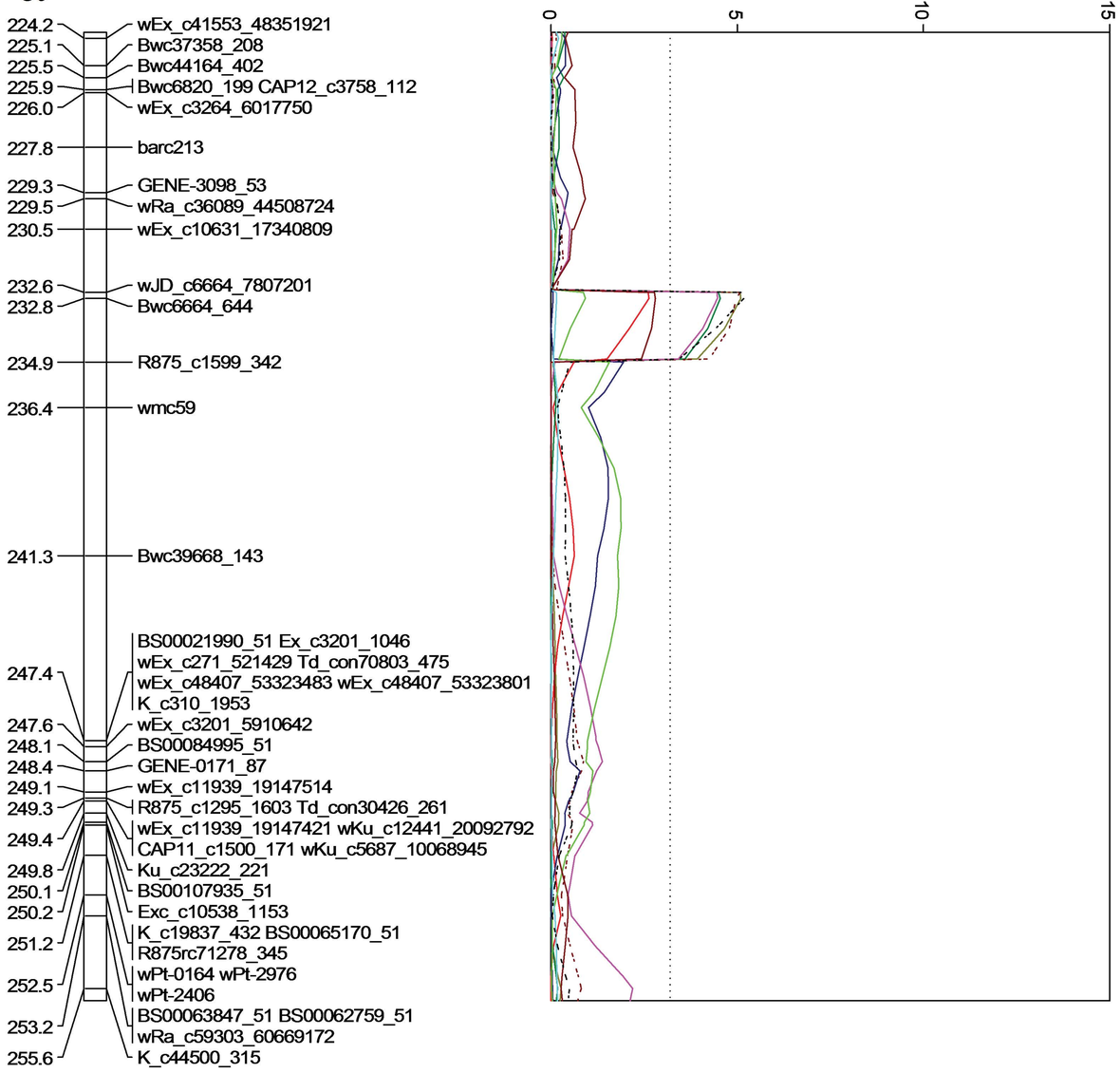
d


SHA3/CBRD x Naxos 7DS
0
1
2
3
4
0.0 wmc438
17.7 gwm1220
22.3 swm10
25.2 Exc_c22419_460 R875_c29314_291
25.3 D_GDS7LZN02FSYZC_227
25.4 K_c92151_216
25.5 wRa_c6894_11980338 K_c94078_311
R875_c1829_321 wBE443044D_Ta_1_1
38.8 gwm16
61.0 wmc405
95.5 barc214
108.0 wExrc68671_67525179
111.2 gwm437
113.5 K_c52429_81
122.5 K_c16416_647
126.9 wEx_c17914_26681837
129.1 R875_c11933_885
130.0 K_c48125_714
130.8 Exc_c4508_1959 Exc_c4508_1007
132.1 Exc_c23328_127
133.9 wmc488
134.9 D_con55386_313
136.4 wEx_c145_285194
138.3 wmc94
141.3 wBE497845D_Ta_1_1


SHA3/CBRD x Naxos
PM Vollebekk 2008
PM Vollebekk 2009
PM Vollebekk 2010
PM Staur 2008
PM Staur 2009
PM Staur 2010
PM Nanjing 2009
PM Beijing 2010
PM Anyang 2010
PM all over mean

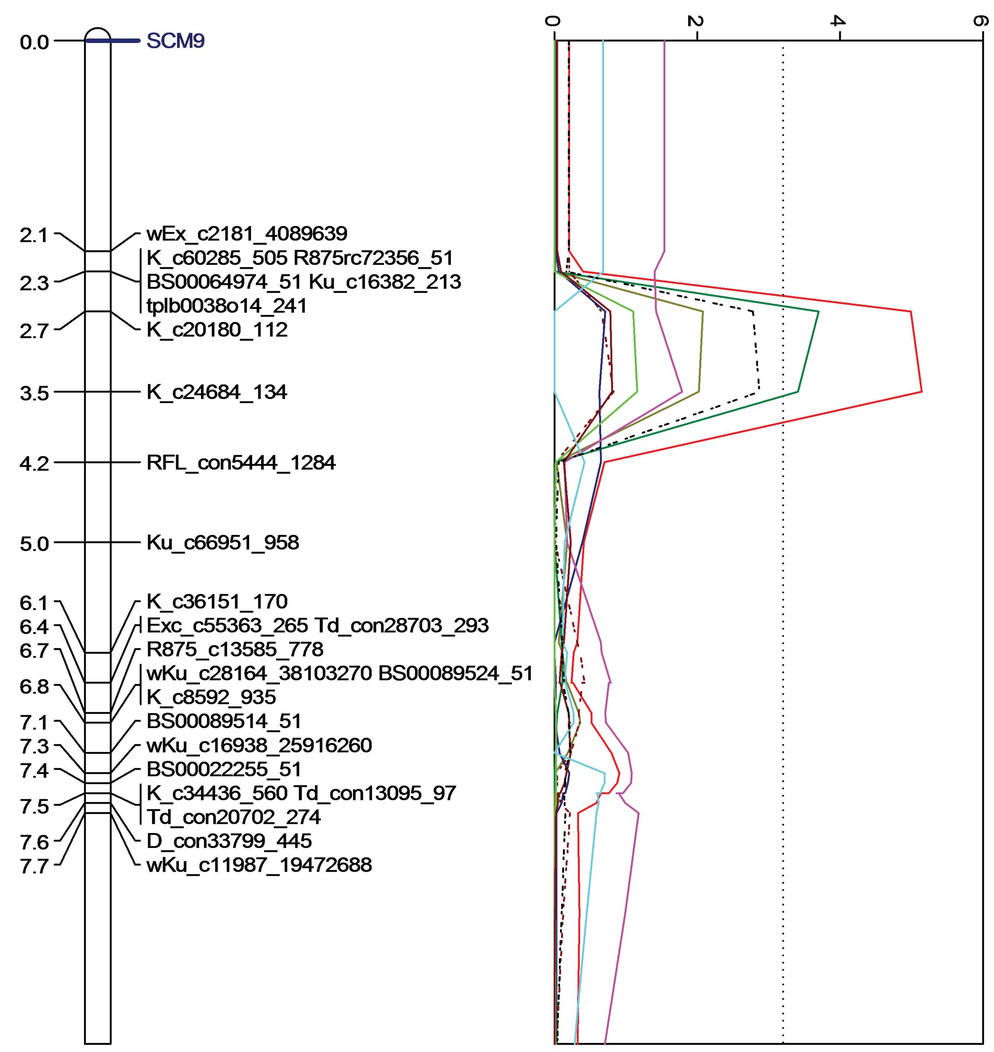
a

SHA3/CBRD x Naxos 1AL



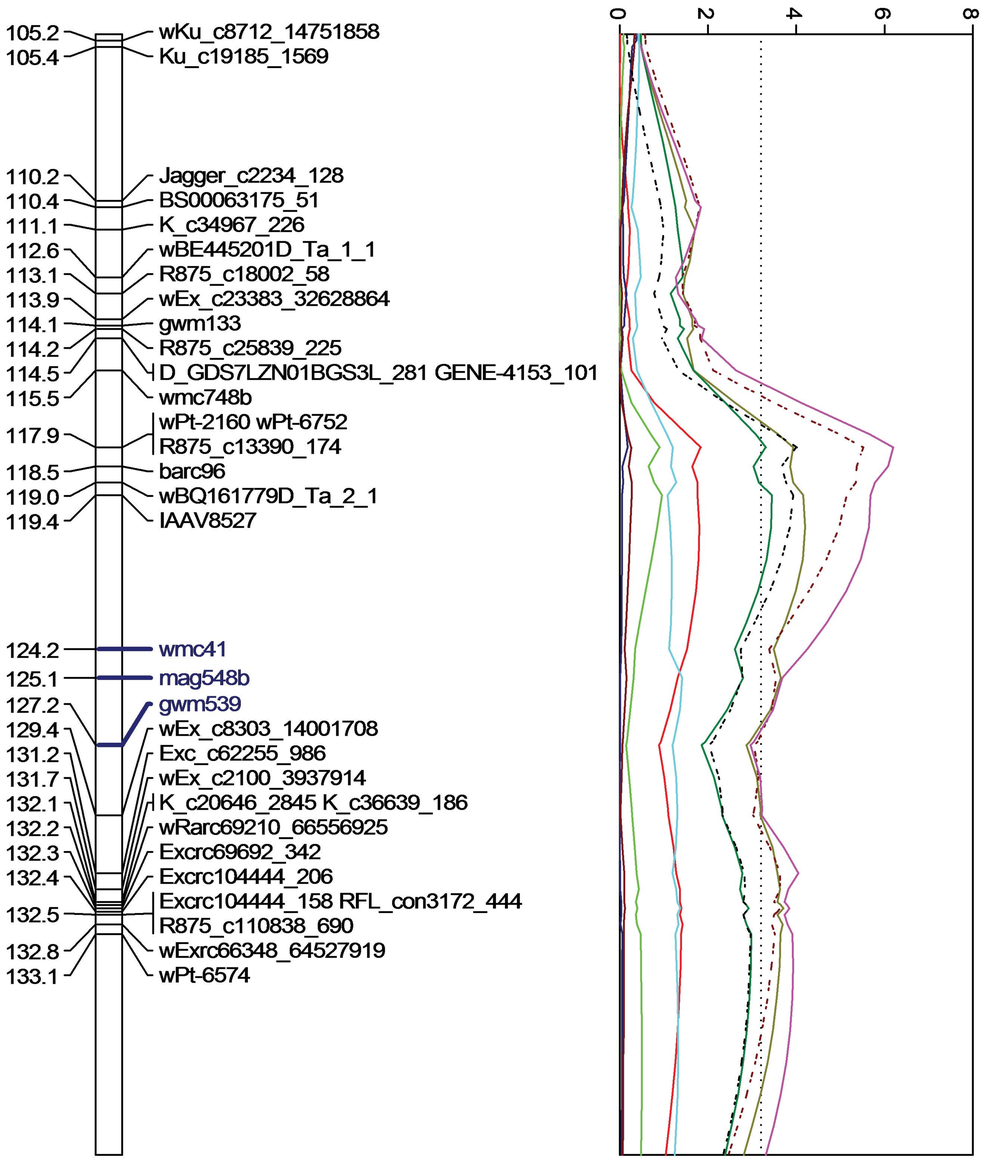
b

SHA3/CBRD x Naxos 1RS



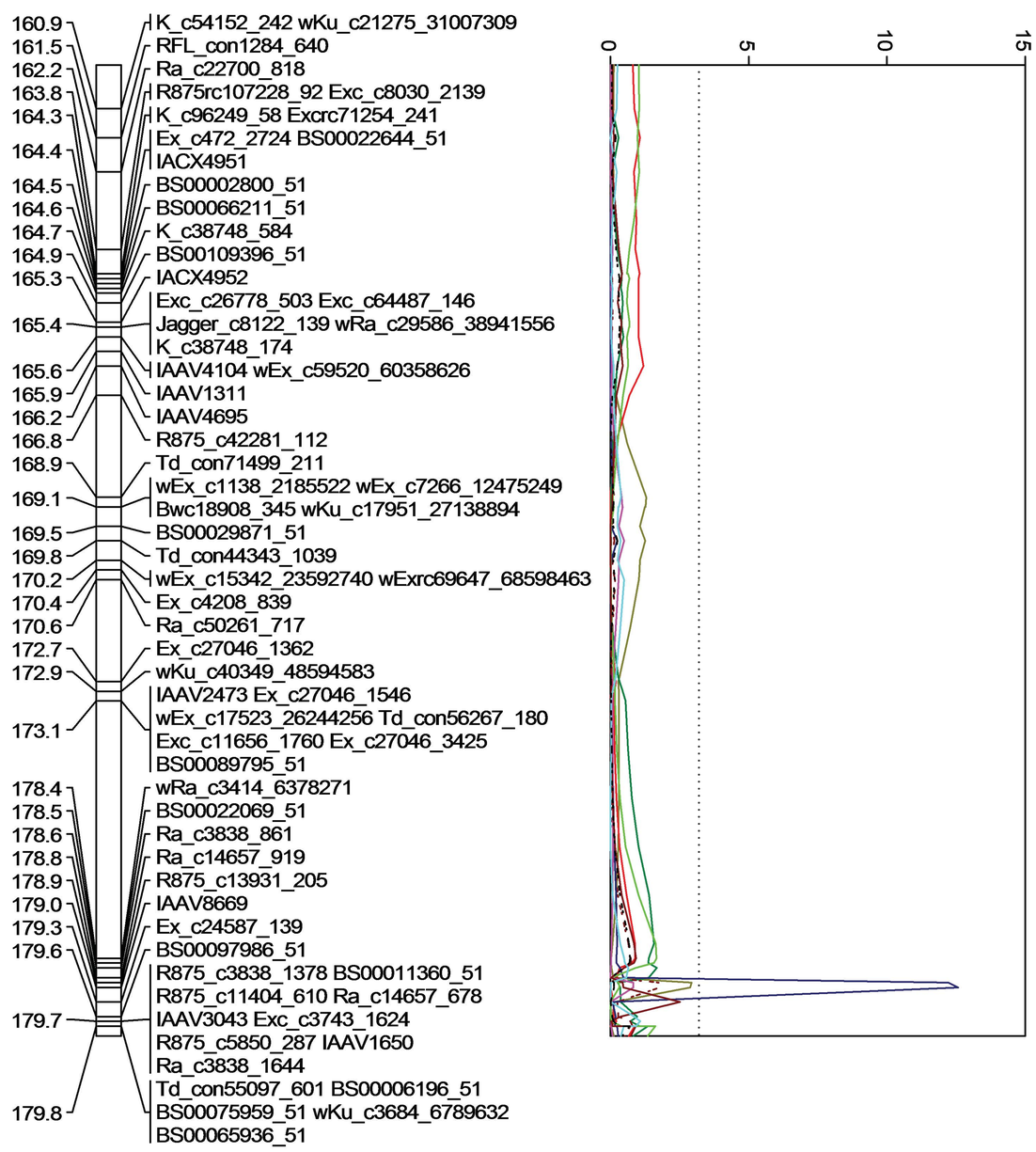
c

SHA3/CBRD x Naxos 2DS



d

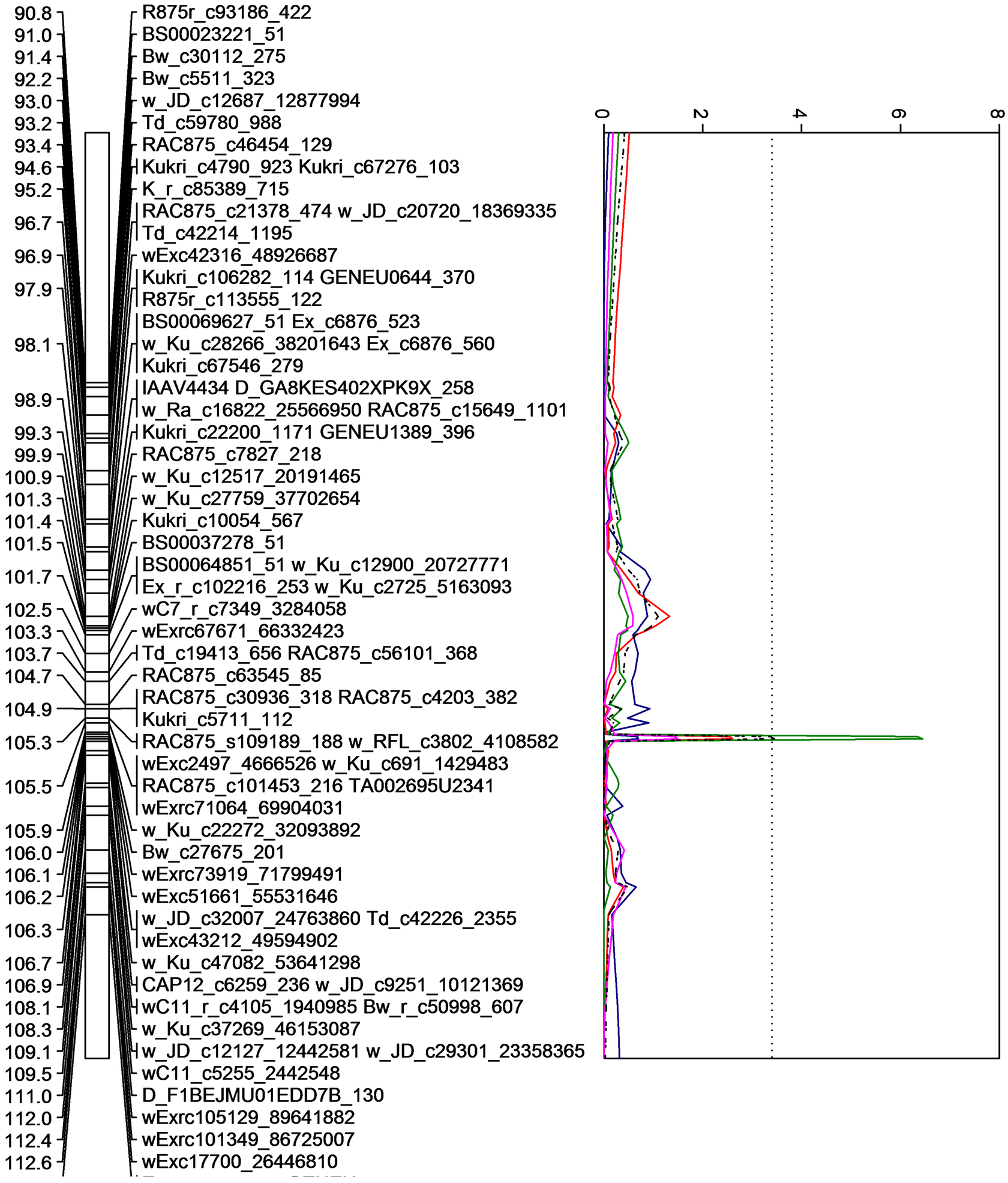
SHA3/CBRD x Naxos 5AL



SHA3/CBRD x Naxos

- PM Vollebekk 2008
- PM Vollebekk 2009
- PM Vollebekk 2010
- PM Staur 2008
- PM Staur 2009
- PM Staur 2010
- PM Nanjing 2009
- PM Beijing 2010
- PM Anyang 2010
- PM all over mean

Soru#1 x Naxos 2BL



Soru#1 x Naxos 3AS