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Identification and Validation of Major Pre-Harvest Sprouting Resistance Loci in Nordic and Exotic Spring Wheat

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Running title: GWAS of pre-harvest sprouting resistance

Abstract

Pre-harvest sprouting (PHS) is a significant concern for global wheat production, which calls the need to identify Quantitative Trait Loci (QTL) and enhance PHS resistance through plant breeding. Genome-wide association studies (GWAS) followed by multivariate analyses and allelic stacking were performed on a spring wheat panel from the Norwegian University of Life Sciences, MASBASIS, a collection of 301 spring wheat genotypes of adapted and exotic (non-adapted) genotypes, grown in 15 field trials over seven years at two locations in Norway and one location in China, characterised by falling number (FN) and/or germination index (GI). By allelic stacking, the most beneficial alleles showed improved PHS resistance up to a high number of beneficial alleles reflecting complementary effects of different genes located on these markers. KnetMiner, a gene identifier and pathway software made for plant genomics, identified several putatively relevant genes with fundamentally different molecular function. The allelic stacking of the identified markers was also significant in an independent set of genotypes from the Norwegian breeding population used for local external validation. Many beneficial alleles are already implemented in the adapted material of MASBASIS and in the validation data as results of year of selection for PHS resistance, but further improvement is sorely needed. As many beneficial markers display complementary effects and are needed for good PHS control, targeted marker-assisted selection (MAS) is a challenge. A strategy of multivariate MAS is therefore suggested for improvement of PHS resistance in plant breeding.

Keywords: *Triticum aestivum* L., pre-harvest sprouting, GWAS allele stacking

Key messages: PHS resistance was improved by increasing the number of favourable QTL alleles up to 14-15 when considered across a wide genetic background of adapted (Nordic) and non-adapted backgrounds, which suggest complementary effects.

Introduction

Pre-harvest sprouting (PHS), the germination of grains within spikes before harvest, is one of the major causes of losses in the bread wheat quality, which adversely impacts global food security (Matilla 2024; Yang et al. 2025). Identifying QTL that contribute to PHS in wheat is crucial for effective wheat breeding to improve PHS resistance. As wheat breeding is time-consuming and PHS provocation in the field is irregular, marker-assisted selection (MAS) at an early stage is an important strategy to increase the frequencies of rare beneficial alleles and maintain high frequencies of beneficial alleles. Advances in spring wheat breeding technologies, enabled by speed breeding and off-season nurseries in combination with genomic tools, have the potential to rapidly improve wheat genetics (Batista et al. 2024). This urgently requires reliable genetic markers for PHS resistance. The revolutionary paradigm shift in molecular tools and knowledge of the wheat genome, which we are currently experiencing, is laying the foundation for identification of reliable genetic markers.

Grain dormancy, the ability to avoid germination until conditions become suitable for germination and seedling establishment, is one of the most critical adaptive strategies for ensuring successful reproduction of plants (Bewley 1997a). At maturity, the seeds of most angiosperms are dormant, and dormancy must be broken before germination can occur (Bewley 1997b). This pause in the plant life cycle allows germination to occur under conditions favourable for growth of the seedling and in a season that provides sufficient time for completion of the next generation. In agriculture crops, there have been a selection against dormancy to ensure rapid uniform germination. However, this has led to the risk of PHS. Germination in the spikes before harvesting is triggered by the combination of humid conditions during and after grain maturation and insufficient seed dormancy (Hull et al. 2024; Jia et al. 2024). Seed dormancy is a complex property involving regulation at different levels (physical, chemical, developmental, physiological and genetic) in interaction with environmental cues (light, temperature, water and nutrients) (Li et al. 2026). The molecular mechanisms underlying induction and retention of dormancy are not yet fully understood.

Germination is strictly co-ordinately controlled, as it must be for a successful germination process and survival of the seedling. In the very first phase of germination there is a strong rapid synthesis of ATP (Obendorf and Marcus 1974), which is needed to cover the high energy demand of all the coordinated molecular processes of germination.

The seed mitochondria are equipped with the proteins needed to produce energy at the onset of germination (Ditz et al. 2026), and mRNAs that were transcribed and stored during seed development are rapidly translated to yield mature proteins within the first few hours of germination (Lewsey et al. 2025; Rajjou et al. 2004). The question is how this coordinated process is regulated.

The importance of reduction-oxidation (redox) in all physiological processes, in all organisms from unicell organisms to plants and animals, are increasingly recognised. Bailly and coworkers were the first introduced the concept “oxidative window” for germination where germination can start via redox signalling when the grains are within the “oxidative window” for germination (Amir et al. 2026; Bailly et al. 2008; Bykova and Igamberdiev 2025). During seed development, reactive oxygen species (ROS) are produced through mitochondrial respiration, photosynthesis, and fatty acid metabolism, and in mature dry seeds ROS are mainly produced by non-enzymatic reactions. During seed germination, ROS production increases, and this controlled ROS production drives vital developmental steps during germination, including cell wall weakening, radicle protrusion, and metabolic reactivation, allowing the seed to break dormancy and initiate germination. The redox signalling are connected with other signalling networks such as calcium (Ca^{2+}) signaling (Cheng et al. 2022; Wu et al. 2020), and it is connected to abscisic acid (ABA) and gibberellic acid (GA_3) signalling (Cheng et al. 2022; Liu et al. 2010), the balance of ABA/GA, and/or the seed sensitivity to ABA and GA (Bradford and Trewavas 1994; Nguyen et al. 2025; Watahiki and Trewavas 2019).

When germination in wheat begins before harvest, it can have deleterious consequences for baking quality and grain quality for other food products. Wheat affected by PHS, above a specific threshold, is downgraded to feed grade, resulting in decreases in the price (Hull et al. 2024). Wheat seeds are usually most dormant at physiological maturity, the time when the flag leaves and spikes turn yellow (Hanft and Wych 1982). Dormancy at this stage is referred to as primary dormancy. The temperature influences the level of dormancy in opposite ways before and after the maturation time. High temperature before the maturation time leads to lower dormancy (Buraas and Skinnes 1985; Jiang et al. 2023; Mares and Mrva 2014; Nakamura et al. 2011; Reddy et al. 1985; Strand 1989), whereas in the after-ripening period, the dormancy reduces faster under warmer than cooler environmental conditions (Obroucheva and Antipova 2000). During post-harvest storage, dormancy of spring wheat may be broken by moist and cool conditions called cold stratification (Mares 1984; Paterson et al. 1989), which simulate natural conditions that the seeds must experience before germination can occur.

The α-amylase activity, a key enzyme in the degradation of starch into smaller sugars, is indirectly measured by the falling number method (FN) (Hagberg 1961; Perten 1964). FN is analysed on the grains after harvest, and ranges from 62 to over 500 seconds (sec), with lower values indicating high α-amylase activity. Another method to assess the level of PHS resistance is the Germination Index (GI), which measures the level of primary dormancy at maturation (Walker-Simmons 1987). The time to germination of individual seeds is recorded, and an index from 0 to

1 is calculated, giving higher weight for early germination. A higher GI value indicates a higher germination rate and lower seed dormancy.

Many quantitative trait loci (QTL) affecting seed dormancy and PHS resistance have been identified, distributed across all the chromosomes in wheat. The most well-characterised PHS-associated chromosomes are located at: 1A (Wang et al. 2024), 2BS (Chao et al. 2010; Chen et al. 2025; Munkvold et al. 2009; Somyong et al. 2014), 3AS (Liu et al. 2008; Liu et al. 2021; Matilla 2024; Osa et al. 2003; Tyagi and Gupta 2012), 3AL, 3BL, and 3DL (Chen et al. 2025; Himi et al. 2024b; Himi et al. 2011; Li et al. 2022; Mcintosh et al. 1989; Rasul et al. 2012; Zhang et al. 2025), 4AL (Chen et al. 2008; Lin et al. 2015; Torada et al. 2005; Torada et al. 2016; Tyagi and Gupta 2012), and 5AL (Liu et al. 2024; Liu et al. 2014). The causal genes underlying some of the significant QTL have been identified (Tai et al. 2021).

Wheat production across different regions is associated with different challenges with respect to dormancy and PHS (Tai et al. 2021). In dry regions, the primary challenge is ensuring consistent seedling emergence within a short window between harvest and sowing. In humid areas, there may be a two-fold challenge: both uniform germination after sowing and avoiding the onset of germination before harvesting. In Norway, rainfall and high humidity during the late growth season are frequent, and the onset of germination before harvesting is the dominating threat to bread wheat production. In Norway, PHS may in certain years result in downgrading of a high proportion of wheat from food to feed quality. Increased self-sufficiency in wheat production for food has been a political goal in Norway for several decades, with a strong focus in recent years, particularly in view of the UN's 17 Sustainable Development Goals. The pioneer, Professor Erling Strand, initiated work on bread wheat quality in Norway through wheat breeding in the 1950s (Strand 1954), a time when Norway had no bread wheat production. Bread wheat quality has remained a continuous focus in the Norwegian breeding program in close collaboration between scientists, farmers, and industry. The breeding for wheat food quality has led to enhanced PHS resistance, as well as improved gluten quality, resulting in a significant increase in Norway's wheat self-sufficiency rate over 30 years, from 0% to as high as 75% in good years. Yet, despite improvements in PHS resistance over those years, PHS causes major variation from year to year. Therefore, wheat cultivars with improved PHS resistance are urgently needed to achieve stable wheat quality and high self-sufficiency in wheat for food in Norway.

The objectives of the current study were to identify and validate major genetic loci for PHS resistance. The panel, called MASBASIS (MAS = Marker-assisted selection BASIS = Basis-collection), consisting of 301 genotypes, including both adapted (Nordic) and non-adapted origin was tested in seven years at two locations in Norway and one in China. GWAS was performed across all genotypes, both adapted and non-adapted. The significant markers were tested in an external collection of Norwegian breeding lines at Graminor AS, used as local independent validation. The present study has identified numerous significant QTL associated with PHS resistance. The frequencies of many significant beneficial alleles were generally higher in the adapted than the non-adapted genotypes of MASBASIS, and the independent local validation data. This both reflect a real-world validation of the

identified markers, and it shows that the strong focus on PHS resistance maintained throughout the Norwegian wheat breeding program have successfully incorporated many beneficial alleles for PHS resistance. Yet, PHS causes serious challenges for wheat production in Norway, and some of the beneficial alleles had low frequencies among the adapted genotypes in MASBASIS and in the local validation data.

Methods

Plant materials and field trials

The NMBU spring wheat panel, MASBASIS, of 301 genotypes used for GWAS in this study represents genetic variations in Norwegian as well as international cultivars and breeding lines (Lin et al. 2023; Lin et al. 2022; Mroz et al. 2023).

Since PHS traits are strongly affected by the environmental conditions and genotype*environmental interactions, many field trials were conducted in different seasons, regions, and locations. Altogether, data from 17 field experiments from 2009 to 2019, comprising two locations in Norway (Vollebekk and Staur) and one location in China (Chengdu) were included. The field trials were performed using α-lattice designs with two replications and plot sizes of 1.5 to 7.5 m^2 in both Vollebekk and Staur research farms. Each line was planted in rows in Chengdu, without randomisation and replication. FN were observed in 15 out of the 17 field trials, 3 field trials had observation of both FN and GI, and 2 field trials were planted only for GI analyses. There were some variations in the number of genotypes included in the different trials.

Mist-irrigation was used for some trials to obtain an optimal provocation of PHS. The mist irrigation was applied to some of the FN trials at Vollebekk in a period from physiological maturity, programmed for a 30-minute duration at the time points 17:00, 18:30, 20:00, and 21:30 on Sundays, Mondays, Thursdays, and Fridays until sprouting was provoked. Among the field trials that received mist irrigation, some fields were later subjected to heavy rainfall, resulting in low FN for most genotypes. Three irrigated field trials therefore had low FN group for most genotypes, one showed a good separation FN, and one had high FN of most genotypes.

A validation set consisting of 889 independent advanced breeding lines of spring wheat from the breeding company Graminor AS (Ridabu, Norway) was used to validate the QTL results of MASBASIS. Altogether, eight field trials were performed, where FN was analysed from six and GI from two field experiments from 2019 to 2022 in four locations in Norway (Vollebekk, Staur, Rød, and Bjørke). The number of genotypes tested in different years and locations ranged from 69 to 309 genotypes per years. There were 177 genotypes in 2019 and 69 genotypes in 2021 for GI at Vollebekk, 221 genotypes at Staur, and 309 at Rød research field for FN in 2019, 69 genotypes at Rød research field for FN in 2020, 267 genotypes at Bjørke and Vollebekk research field in 2021, and 111 genotypes at Bjørke in 2022. All these genotypes are considered adapted to the Norwegian climate. The field trials were performed using an α-lattice design, with two replications per field trial, with randomisation within replicate. The

validation set was grown under natural conditions with a plot size of 1.5*5 m. Data on the daily mean, minimum and maximum temperature, precipitation and relative humidity were collected from nearby weather stations to the field trials (Data source: Landbruks Meteorologisk Tjeneste).

Germination Index (GI)

Assessment of GI, which measures the level of primary dormancy, was performed at the yellow ripening stage as previously described (Begum et al. 2025) on four field trials at Vollebekk. Briefly, two replicates of 25 mature and dry seeds were laid out in petri dishes with 5 ml of distilled water and allowed to germinate in an incubator at 19°C. The germination period was 14 days, with 16 hours of light and 8 hours of darkness. GI experiments for Chengdu trials were performed at 20°C temperature using dry seeds harvested 35-40 days after anthesis. GI was calculated using the formula described (Walker-Simmons 1987) with slight modifications. Details of this methodology are presented previously (Begum et al. 2025).

Falling Number (FN)

The harvested grain samples were analysed by FN (Perten 1964). The FN means are adjusted due to block and rep effects. Due to the correction, although 62 seconds is the minimum value of FN of the individual sample, some adjusted mean data may have values below that limit.

Statistical analysis of the phenotype data

For each trial, the least square means (LSMeans) of FN and GI were calculated within each field trial by PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using genotypes as a fixed effect, while blocks and replications within block were random effects. For the trial in Chengdu, where biological replications were not available, means over the technical replications were used to estimate LSmeans. LSmeans were calculated for FN across all 15 field trials with FN observations by PROC MIXED model, considering the 15 different environments as random effects and the line as the fixed effects. Similarly, LSmeans of GI were analysed by PROC MIXED across all 5 field trials where GI was observed using the 5 environments as random effects and line as fixed effects.

Data analyses of phenotypic observations were performed in RStudio. Scatter plots with pearson correlations and histograms were made using "*pairspanel()*" from "psych", PCA using "fviz_pca_biplot()".

Genotypic analysis

The NMBU spring wheat panel (MASBASIS) was genotyped with both Affymetrix 35K (Allen et al., 2017) and Trait Genetics 25K single-nucleotide polymorphism (SNP) wheat chips, and the SNP marker data are described in detail previously (Lin et al. 2022; Nannuru et al. 2022). A total of 14,582 SNP markers for 35K and 18,577 SNP markers for 25K were scored as polymorphic and used in GWAS. An artificial chromosome 22 was created for unmapped markers. The population of advanced breeding lines from the Norwegian breeding lines at Graminor, used for validation, was analysed only by the 25K chip.

Association analysis

GWAS was applied both on data from the individual field trials and on groups of trials defined according to the average FN: high FN (mean FN>300 sec), medium FN (mean FN 200-300 sec), low FN (mean FN < 200 sec). GWAS was performed using "FarmCPU" model from "GAPIT3" package of R (Wang and Zhang 2021). Significant markers were validated by Manhattan plots, by significant observed vs expected -log*P* value distributions, and by quantile-quantile (Q-Q) plots drawn in R using the package called "qqman" (Turner 2018). The associations between markers and PHS traits were considered significant when the False Discovery Rate (FDR) adjusted *p*-value was ≤0.05.

After filtering the significant markers in R, a BLASTn (Nucleotide Basic Local Alignment Search Tool) search was performed against the International Wheat Genome Sequencing Consortium database (IWGSC RefSeq v1.0 all chromosomes) to find the physical positions of unmapped markers (SNPs and SSR markers) showing significant trait associations. Only the best hits with over 99% identity and 100% marker coverage were selected from BLASTn search. The probe sequences were obtained from "Grain Genes: A data base for TRiticae and Avena" (https://wheat.pw.usda.gov/GG3/) digital platform, and BLASTn search was performed on "Wheat@URGI" (https://wheat-digital platform, and BLASTn was performed on "Wheat@URGI" (https://wheat-urgi.versailles.inra.fr) online portal. Markers were considered in a QTL when they showed R2>0.6 in linkage disequilibrium (LD), which was performed in TASSEL (v.5.2.44). The QTL were compared with published QTL and considered similar if they were found within 30 Mbp.

KnetMiner for identification of genes and pathways of the significant genetic markers

The QTL identified by GWAS was analysed by KnetMiner (Hassani-Park et al. 2021) to identify candidate genes located. KnetMiner is a plant software platform that generate gene networks and identify candidate genes, pathways, and gene ontologies based on knowledge mining approaches. KnetMiner uses IWGSC RefSeq v1.0 for wheat, loaded via Ensembl Plants. The positions of the peak markers in each QTL were loaded, and a range on each side on +/- 2 Mbp was applied on each side of the marker position. A set of keywords were used in KnetMiner to ensure that genes relevant for PHS are identified. The following keywords were used: PHS OR PHST OR "pre-harvest sprouting" OR dormancy OR "falling number" OR "alpha-amylase" OR "TaMFT" OR "Vp-1" OR "abscisic acid" OR vivipary.

Multivariate analyses

Multivariate data analyses were performed on the field trials with optimal PHS provocation using the markers as input in the analyses. The two alternative alleles for each marker were binarized and transformed to +1 if an allele is present, -1 if absent, and 0 if unknown. Explorative analyses was performed by Principal component analyses (PCA) and supervised multivariate analyses by Partial Least Squares regression (PLS) (Wold et al. 1983) using FN or GI as responses. Whereas PCA reflect the variance structure within a data set, PLS identifies the covariance structure

between the input data (genetic markers) and the response (FN or GI). PLS thereby reflect how the gene markers, and their correlation structure, in combinations, affect the phenotype. Three sets of models were applied: across all genotypes, for only the non-adapted genotypes, and for only the adapted genotypes. Feature selection in PLS was applied by the Jackknife procedure (Martens and Martens 2000), which validate the impact of the individual gene marker alleles by their contribution to the multivariate model. The multivariate analyses were performed in RStudio version 2025.05.0 and R version 4.5.0. PCA was performed by the function *prcomp* in the package *stats* https://cran.r-project.org/web/packages/stats/index.html), PLS was performed using the function *plrs* of the R package *pls* https://cran.r-project.org/web/packages/pls/index.html).

Allele stacking

Allele stacking was performed to study the effects on FN and GI of combining beneficial alleles of the most important QTL from the multivariate analysis. The markers chosen for allelic stacking were 34 marker alleles that were significantly positively associated with PHS resistance, observed by FN or by GI, considering Jackknife in PLS and with PLS loadings. The analysis was performed separately across all genotypes, among the non-adapted genotypes and among the adapted genotypes. Among these 34 markers, 23 markers were available on the validation data, where only one (25K) of the two ships (25K and 35K) were applied. Regression analyses were conducted in the allelic stacking analyses of each field trial with the number of beneficial alleles as one input variable and the phenotype, FN or GI, as response.

Results

Phenotypic evaluation

A complete presentation of the phenotypes from all field trials of MASBASIS are presented in Table S1. The field trials were grouped according to the average FN (Table 1) showed high mean FN (mean FN>300 sec) for the field trials n1, n8, n10, n13, n15, n18, n20, medium mean FN (mean FN 200-300 sec) for the field trials: n2, n6, n7, and low mean FN (mean FN < 200 sec) for the field trials n3, n4, n11, n14, n19. All data with GI was considered to represent a common GI group.

Correlation matrix plot between the different field trials (Fig S2) shows close correlations within and among the field trials in the groups high and medium FN, and field trial n19 from the low FN group, whereas the other field trials in the low FN group showed low intercorrelations and low correlations to the other trials. The FN field trials reflecting most optimal variations in PHS resistance were the FN-field trials n2, n6, n7, n19. For GI, all 5 GI-field trials show positive intercorrelation, most strongly among those grown in Norway.

As GI is observed on grains at yellow maturation, while FN from the same field experiments is observed after harvesting, GI and FN from these field trials represent different environmental impact with respect to PHS provocation. Therefore, although GI and FN at Vollebekk in 2015 (n5 GI and n6 FN), 2016 (n9 GI and n10 FN), and

2017 (n12 GI and n13 FN) are from the same field experiment, the GI and FN data from these field trials are categorised to represent different environments and assigned as different field trial numbers. The number of genotypes included in each field trials varied, where 84 common genotypes were included in all 15 FN field trials, and 119 common genotypes were included in all 5 GI field trials.

Table 1. Overview of the field trials for phenotypic data used for the GWAS on the MASBASIS panel. The names of the field trials describe the location (v, s or c for Vollebekk, Staur and Chengdu, respectrively), phenotype (FN/GI), phenotype group (h, m, and l for high mean FN (>300 sec), medium mean FN (200-300 sec), and low mean FN (>200 sec), respectively), year of growth, trial number (1-20), and mist irrigation if used (M).

Trial no	Trial code	Location	Year	Trait measured	Mist-irrigated	Phenotype range (mean in parenthesis)
n1	vFNh09**n1**	Vollebekk	2009	FN	No	114.92-425.05 (307.61)
n2	sFNm09**n2**	Staur	2009	FN	No	66.35-395.81 (244.50)
n3	vFNl12**n3**M	Vollebekk	2012	FN	Yes	62-203.44 (78.47)
n4	vFNl15**n4**M	Vollebekk	2015	FN	Yes	62-252.31 (107.27)
n6	vFNm15**n6**	Vollebekk	2015	FN	No	62.52-419.04 (209.95)
n7	vFNm16**n7**M	Vollebekk	2016	FN	Yes	62-440.32 (249.55)
n8	sFNh16**n8**	Staur	2016	FN	No	96.56-546.20 (320.71)
n10	vFNh16**n10**	Vollebekk	2016	FN	No	62-463.26 (335.98)
n11	sFNl17**n11**	Staur	2017	FN	No	62-259.25 (78.39)
n13	vFNh17**n13**	Vollebekk	2017	FN	No	62-496.99 (368.78)
n14	vFNl17**n14**M	Vollebekk	2017	FN	Yes	62-153.44 (65.75)
n15	sFNh18**n15**	Staur	2018	FN	No	100.18-575.96 (395.98)
n18	vFNh18**n18**M	Vollebekk	2018	FN	Yes	70.02-494.14 (330.85)
n19	sFNl19**n19**	Staur	2019	FN	No	62-356.38 (191.42)
n20	vFNh19n**20**	Vollebekk	2019	FN	No	62-447.93 (320.99)

n5	vGI15**n5**	Vollebekk	2015	GI	No	0.05-0.72 (0.32)
n9	vGI16**n9**	Vollebekk	2016	GI	No	0.00-0.80 (0.34)
n12	vGI17**n12**	Vollebekk	2017	GI	No	0.07-0.70 (0.36)
n16	vGI18**n16**	Vollebekk	2018	GI	No	0.06-0.86 (0.50)
n17	cGI18**n17**	Chengdu	2018	GI	No	0.08-0.86 (0.56)

The FN was generally higher for the adapted genotypes compared with the non-adapted genotypes. Histogram of all field trials are provided in Fig S1 and histogram of means across the four field trials with most optimal PHS provocation (n2, n6, n7, n19) are displayed in Fig 1, separately for the non-adapted and adapted genotypes.

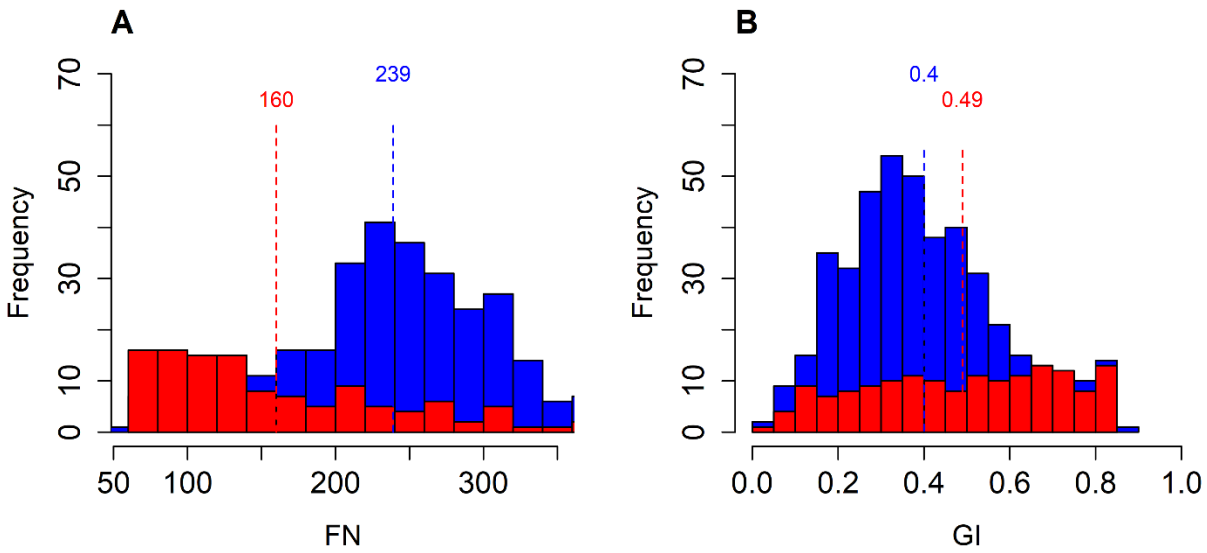


Fig. 1. Histograms. (A) Histograms of FN of MASBASIS of common genotypes included in all four FN-field-trials reflecting the most optimal PHS provocation (n2, n6, n7 and n19): 32 non-adapted genotypes (red) and 86 adapted genotypes (blue). (B) Histograms of GI of all GI-field-trials (n5, n9, n12, n16, and n17) for common genotypes: 31 non-adapted genotypes (red) and 87 adapted (blue). The mean FN and mean GI among the adapted and the non-adapted genotypes are visualised by vertical lines, and the numeric mean is printed.

The validation data set (Table S2), which was field trials observed by FN from the independent collection of the Norwegian wheat breeding program, reflected optimal PHS provocations in two field trials from 2019, grown at Staur and Rød, respectively (Fig. S3). GI was observed in the field trials grown at Vollebekk in 2019 and 2021.

Genome-wide association analysis

GWAS of MASBASIS with the phenotypes FN and GI using the 25K SNP chip and 35K SNP chip are presented as Manhattan plots on group level in Fig. S4-S5, QQ plots performed on the groups in Fig. S6-S7, and GWAS applied on the data from each field trials and GWAS applied on the group level are summarised in Table S3. The significant markers assigned as QTL and the peak markers in each QTL are presented in Table 2 with additional information in Table S4. The SNPs of the validation data using the 25K SNP are presented in Table S5.

Table 2. Summary of the significant markers QTL and peak markers detected by GWAS using both 25K and 35K SNP chips. The position of the peak markers in each QTL are presented by their position (Mbp) with with a range +/- 2 Mbp. GWAS was applied both on the groups defined by mean FN: high (>300 sec), medium (200-300 sec) and low mean FN (<200 sec), and on the individual trials n1-n20 (defined in Table 1). Abbreviations: SNP: single nucleotide polymorphism, megabasepair (Mbp) = 1 mill Basepair.

Chromo-some	QTL	Markers	25K	35K	Mbp	Position +/- 2 Mbp	-logP	Sign. in groups	Sign. in individual trials
1A	*QFN.nmbu_masbasis-1A.1*	*AX-94735017*		*x*	9.58	7.58-11.58	9.44	hFN	n1, n2
		TA005766.0499	*x*		11.90	9.9-13.9	4.56	hFN	
	QFN.nmbu_masbasis-1A.2	*AX-94677073*	x		22.29	20.29-24.29	4.90	mFN	n7
	QFN.nmbu_masbasis-1A.3	*Kukri_c7436_2259*	*x*		26.96	24.96-28.96.	5.04	mFN	
	QFN.nmbu_masbasis-1A.4	*GENE-0032_895*	*x*		111.13	109.13-113.13	8.38	hFN	n1, n20, n13
	QGI.nmbu_masbasis-1A.1	*wsnp_Ex_c35563_43690084*	*x*		377.43	375.43-379.43	5.87	GI	
	QFN.nmbu_masbasis-1A.5	*AX-95187774*	*x*		550.44	548.44-552.44	6.22	lFN	
1B	*QFN.nmbu_masbasis-1B.1*	*AX-95245523*		*x*	15.75	13.75-17.75	6.24	lFN	n6
	QFN.nmbu_masbasis-1B.2	*AX-94661034*		*x*	80.92	78.92-82.92	5.48, 5.33	hFN, mFN	
	QFN.nmbu_masbasis-1B.3	*Kukri_rep_c69810_502*	*x*		469.98	467.98-469.98	5.37	lFN	
	QFN.nmbu_masbasis-1B.4	*wsnp_Ex_c1493_2860766*	*x*		670.18	668.18-672.18	4.57	hFN	
1RS	*QFN.nmbu_masbasis-1RS.1*	*Tdurum_contig47083_278*	*x*		10.10	8.10-12.10	8.03, 4.84	hFN, mFN	
		BS00004903_51	*x*		11.63	9.63-13.63	6.03	hFN	
		RAC875_rep_c70613_78	*x*		11.93	9.93-13.93	6.03	hFN	
		BobWhite_c28295_256	*x*		14.85	12.85-16.85	5.01	hFN	
2A	*QFN.nmbu_masbasis-2A.1*	*AX-95198970*		*x*	17.31	15.31-19.31	5.20	hFN	

	QFN.nmbu_masbasis-2B.1	*AX-95017965*		*x*	19.07	17.07-21.07	13.09	lFN	
	QFN.nmbu_masbasis-2B.2	*AX-158562351*	*x*		41.42	39.42-43.42	4.77	hFN	
	QFN.nmbu_masbasis-2B.3	*wsnp_RFL_Contig3917_4326857*	*x*		743.75	741.75-745.75	6.01	lFN	n15, n19
2D	*QFN.nmbu_masbasis-2D.1*	*AX-95193576*		*x*	635.95	633.95-637.95	5.31	mFN	
3A	*QFN.nmbu_masbasis-3A.1*	*BS00092728_51*	*x*		1.31	0-3.31	7.72	hFN	n8
		AX-94993536		*x*	6.69	4.69-8.69	5.47	mFN	
3B	*QGI.nmbu_masbasis-3B.1*	*BobWhite_c20876_475*	*x*		417.26	415.26-419.26	5.28	GI	n8, n14
		AX-158563556	*x*		474.40	472.40-476.40	7.72	hFN	n1
	QFN.nmbu_masbasis-3B.2	*wsnp_Ex_c6245_10887043*	*x*		669.43	667.43-671.43	6.16	hFN	n10
	QFN.nmbu_masbasis-3B.3	*AX-94785160*		*x*	742.26	740.26-744.26	5.41	mFN	n7, n8
3D	*QGI.nmbu_masbasis-3D.1*	*AX-94791751*		*x*	571.23	569.23-573.23	6.42	GI	
	QFN.nmbu_masbasis-3D.1	*AX-95120609*		*x*	571.23	569.23-573.23	5.50	hFN	
4A	*QGI.nmbu_masbasis-4A.1*	*PM19_A1_PCR_114*		*x*	602.97	600.97-604.97	6.59	GI	n17
	QFN.nmbu_masbasis-4A.1, QGI.nmbu_masbasis-4A.1	*BS00072025_51*	*x*		605.66	603.66-607.66	6.07	GI	n9, n14
	QGI.nmbu_masbasis-4A.2	*BobWhite_c10610_149*	*x*		686.38	684.38-688.38	8.23	GI	n9, n16
4B	*QFN.nmbu_masbasis-4B.1*	*AX-94420304*		*x*	322.75	320.75-324.75	16.25	lFN	n6, n13, n15, n18, n19, n20
	QGI.nmbu_masbasis-4B.1	*AX-110791800*	*x*		535.25	531.25-537.25	7.33	GI	n16
4D	*QFN.nmbu_masbasis-4D.1*	*AX-94680941*	*x*		439.13	437.13-441.13	4.73	mFN	
5A	*QFN.nmbu_masbasis-5A.1*	*AX-94419772*		*x*	445.19	443.19-447.19	4.88	lFN	n15, n19
	QFN.nmbu_masbasis-5A.2	*AX-158558875*	*x*		535.75	533.75-537.75	5.47	mFN	
	QGI.nmbu_masbasis-5A.1	*AX-158584188*	*x*		664.27	662.27-666.27	8.01	GI	
5B	*QFN.nmbu_masbasis-5B.1*	*AX-158533842*	*x*		580.69	578.69-582.69	7.21, 8,48	hFN, lFN	
5D	*QGI.nmbu_masbasis-5D.1*	*AX-94655148*		*x*	612.67	610.67-614.67	5.36	GI	
	QGI.nmbu_masbasis-5D.2	*AX-94806248*		*x*	562.65	560.65-564.65	4.69	GI	
6A	*QGI.nmbu_masbasis-6A.1*	*AX-95246205*		*x*	584.94	582.94-586.94	5.00	GI	

6D	*QFN.nmbu_masbasis-6A.1*	*AX-94634087*	*x*	612.33	610.33-614.33	5.43 mFN	n10
	QFN.nmbu_masbasis-6D.1	*wsnp_Ex_c37749_45436366*	*x*	178.68	176.68-180.68	5.35 mFN	
7A	*QFN.nmbu_masbasis-7A.1, QGI.nmbu_masbasis-7A.1*	*BS00105531_51*	*x*	717.04	715.04-719.04	4.48 mFN	n9
	QFN.nmbu_masbasis-7A.1	*AX-94841408*	*x*	726.91	724.91-728.91	8.39, 8.15 hFN, mFN	n6, n7
	QFN.nmbu_masbasis-7A.2	*tplb0045c05_547*	*x*	736.43	734.43-738.43	13.80 hFN, lFN	n8, n13, n15, n19, n20
7B	*QFN.nmbu_masbasis-7B.1*	*CAP12_c1816_325*	*x*	21.67	19.67-23.67	7.16 mFN	
	QFN.nmbu_masbasis-7B.2	*AX-158544142*	*x*	40.44	38.44-42.44	5.94 hFN	
	QGI.nmbu_masbasis-7B.1	*AX-94505411*	*x*	617.57	615.57-619.57	4.95 GI	n17
	QFN.nmbu_masbasis-7B.3	*AX-95235606*	*x*	630.66	628.66-632.66	5.15 lFN	
	QFN.nmbu_masbasis-7B.4	*AX-158592462*	*x*	730.23	728.23-732.23	5.31 mFN	n7, n10
7D	*QFN.nmbu_masbasis-7D.1*	*AX-95155766*	*x*	111.37	109.37-113.37	6.73 mFN	
	QFN.nmbu_masbasis-7D.2	*AX-95157119*	*x*	585.15	583.15-587.15	4.78 mFN	n8, n20
	QGI.nmbu_masbasis-7D.3	*AX-94401880*	*x*			5.30 GI	

Table 2B. QTL presented in Table 2A aligned with published regions considered similar if they are within +/-30 Mbp threshold for overlap.

Chromo-some	**QTL**	**Most significant marker**	**References**
1A	*QFN.nmbu_masbasis-1A.1*	*AX-94735017*	(Lin et al. 2016; Lohwasser et al. 2013; Mohan et al. 2009; Rabieyan et al. 2022; Zhou et al. 2017; Zuo et al. 2019)
		TA005766.0499	(Lin et al. 2016; Lohwasser et al. 2013; Mohan et al. 2009; Rabieyan et al. 2022; Zhou et al. 2017; Zuo et al. 2019)
	QFN.nmbu_masbasis-1A.2	*AX-94677073*	(Jiang et al. 2022; Rabieyan et al. 2022; Tai et al. 2021; Zhou et al. 2017; Zuo et al. 2019)

	QFN.nmbu_masbasis-1A.3	*Kukri_c7436_2259*	(Jiang et al. 2022; Rabieyan et al. 2022; Tai et al. 2021; Zhou et al. 2017; Zuo et al. 2019)
	QFN.nmbu_masbasis-1A.4	*GENE.0032_895*	
	QGI.nmbu_masbasis-1A.1	*wsnp_Ex_c35563_43690084*	(Albrecht et al. 2015; Börner et al. 2018)
	QFN.nmbu_masbasis-1A.5	*AX-95187774*	(Zhu et al. 2019)
1B	*QFN.nmbu_masbasis-1B.1*	*AX-95245523*	(Begum et al. 2025; Farrell et al. 2013; Tang et al. 2017; White et al. 2022)
	QFN.nmbu_masbasis-1B.2	*AX-94661034*	(Begum et al. 2025)
	QFN.nmbu_masbasis-1B.3	*Kukri_rep_c69810_502*	(Cao et al. 2016)
	QFN.nmbu_masbasis-1B.4	*wsnp_Ex_c1493_2860766*	(Martinez et al. 2018)
1RS	*QFN.nmbu_masbasis-1RS.1*	*Tdurum_contig47083_278*	(Begum et al. 2025; Farrell et al. 2013; Tang et al. 2017; White et al. 2022)
		BS00004903_51	(Begum et al. 2025; Farrell et al. 2013; Tang et al. 2017; White et al. 2022)
		RAC875_rep_c70613_78	(Begum et al. 2025; Farrell et al. 2013; Tang et al. 2017; White et al. 2022)
		BobWhite_c28295_256	(Begum et al. 2025; Farrell et al. 2013; Tang et al. 2017; White et al. 2022)
2A	*QFN.nmbu_masbasis-2A.1*	*AX-95198970*	(Martinez et al. 2018; Mohan et al. 2009; Rabieyan et al. 2022; Zhu et al. 2019; Zhu et al. 2016)
2B	*QFN.nmbu_masbasis-2B.1*	*AX-95017965*	(Lin et al. 2016; Rabieyan et al. 2022; Zhu et al. 2019)
	QFN.nmbu_masbasis-2B.2	*AX-158562351*	Lin et al. 2016; Rabieyan et al. 2022; Zhu et al. 2019)

	QFN.nmbu_masbasis-2B.3	*wsnp_RFL_Contig3917_4326857*	(Begum et al. 2025; Lin et al. 2016; Lohwasser et al. 2013; Munkvold et al. 2009; Tai et al. 2021)
2D	*QFN.nmbu_masbasis-2D.1*	*AX-95193576*	(Jiang et al. 2022; Lin et al. 2016; Zhu et al. 2019)
3A	*QFN.nmbu_masbasis-3A.1*	*BS00092728_51*	(Cao et al. 2016; Dhariwal et al. 2021; Himi et al. 2024a; Lin et al. 2016; Liu et al. 2008; Liu et al. 2015b; Liu et al. 2010; Martinez et al. 2018; Mohan et al. 2009; Mori et al. 2005; Moullet et al. 2022; Nakamura et al. 2011; Osa et al. 2003; Shao et al. 2018; Zhu et al. 2019; Zuo et al. 2019)
		AX-94993536	(Cao et al. 2016; Dhariwal et al. 2021; Himi et al. 2024a; Lin et al. 2016; Liu et al. 2008; Liu et al. 2015b; Liu et al. 2010; Martinez et al. 2018; Mohan et al. 2009; Mori et al. 2005; Moullet et al. 2022; Nakamura et al. 2011; Osa et al. 2003; Shao et al. 2018; Zhu et al. 2019; Zuo et al. 2019)
3B	*QGI.nmbu_masbasis-3B.1*	*BobWhite_c20876_475*	(Chang et al. 2010a; Chang et al. 2010b; Jiang et al. 2022; Kumar et al. 2015; Mares et al. 2009; Zhou et al. 2017)
	QFN.nmbu_masbasis-3B.1	*AX-158563556*	(Cabral et al. 2014; Jaiswal et al. 2012; Jiang et al. 2022; Tai et al. 2021; Zhou et al. 2017)
	QFN.nmbu_masbasis-3B.2	*wsnp_Ex_c6245_10887043*	(Cabral *et al.*, 2014; Liu *et al.*, 2024)
	QFN.nmbu_masbasis-3B.3	*AX-94785160*	(Arif et al. 2012; Begum et al. 2025; Groos et al. 2002; Jaiswal et al. 2012; Lin et al. 2016; Zhou et al. 2017; Zhu et al. 2019)

3D	*QGI.nmbu_masbasis-3D.1*	*AX-94791751*	(Dhariwal et al. 2021; Fofana et al. 2009; Groos et al. 2002; Himi et al. 2024a; Himi et al. 2011; Jiang et al. 2022; Lang et al. 2021; Lin et al. 2016; Zuo et al. 2019)
	QGI.nmbu_masbasis-3D.1	*AX-95120609*	
4A	*QGI.nmbu_masbasis-4A.1*	*PM19_A1_PCR_114*	(Barrero et al. 2015; Shorinola et al. 2017; Shorinola et al. 2016)
	QFN.nmbu_masbasis-4A.1, QGI.nmbu_masbasis-4A.1	*BS00072025_51*	(Barrero et al. 2015; Begum et al. 2025; Dallinger et al. 2024; Kulwal et al. 2012; Kumar et al. 2015; Lin et al. 2016; Martinez et al. 2018; Zhu et al. 2019; Zhu et al. 2016; Zuo et al. 2019)
	QGI.nmbu_masbasis-4A.2	*BobWhite_c10610_149*	(Begum et al. 2025; Lohwasser et al. 2013; Zuo et al. 2019)
4B	*QFN.nmbu_masbasis-4B.1*	*AX-94420304*	
	QFN.nmbu_masbasis-4B.2	*AX-110791800*	(Dhariwal et al. 2021; Jaiswal et al. 2012; Jiang et al. 2022; Mori et al. 2005; Wang et al. 2019; Zhu et al. 2019)
4D	*QFN.nmbu_masbasis-4D.1*	*AX-94680941*	(Dhariwal et al. 2021; Jiang et al. 2022)
5A	*QFN.nmbu_masbasis-5A.1*	*AX-94419772*	(Martinez et al. 2018; Zuo et al. 2019)
	QFN.nmbu_masbasis-5A.2	*AX-158558875*	(Dhariwal et al. 2021; Martinez et al. 2018)
	QGI.nmbu_masbasis-5A.1	*AX-158584188*	(Jiang et al. 2022; Zuo et al. 2019)
5B	*QFN.nmbu_masbasis-5B.1*	*AX-158533842*	(Jiang et al. 2022; Liu et al. 2015b; Singh et al. 2010; Tai et al. 2021; Zhu et al. 2019; Zuo et al. 2019)
5D	*QGI.nmbu_masbasis-5D.1*	*AX-94655148*	(Jiang et al. 2022; Kumar et al. 2015; Martinez et al. 2018; Tai et al. 2021; Zuo et al. 2019)

	QGI.nmbu_masbasis-5D.2	*AX-94806248*	(Börner et al. 2018; Jiang et al. 2022; Kulwal et al. 2012; Zhu et al. 2019)
6A	*QGI.nmbu_masbasis-6A.1*	*AX-95246205*	(Kumar et al. 2009; Zuo et al. 2019)
	QFN.nmbu_masbasis-6A.1	*AX-94634087*	(Kumar et al. 2009)
6D	*QFN.nmbu_masbasis-6D.1*	*wsnp_Ex_c37749_45436366*	
7A	*QFN.nmbu_masbasis-7A.1, QGI.nmbu_masbasis-7A.1*	*BS00105531_51*	
	QFN.nmbu_masbasis-7A.1	*AX-94841408*	(Lohwasser et al. 2013; Martinez et al. 2018)
	QFN.nmbu_masbasis-7A.2	*tplb0045c05_547*	(Lohwasser et al. 2013; Martinez et al. 2018)
7B	*QFN.nmbu_masbasis-7B.1*	*CAP12_c1816_325*	(Jiang et al. 2022; Rabieyan et al. 2022; Tai et al. 2021; Zuo et al. 2019)
	QFN.nmbu_masbasis-7B.2	*AX-158544142*	(Jiang et al. 2022; Tai et al. 2021; Zuo et al. 2019)
	QGI.nmbu_masbasis-7B.1	*AX-94505411*	(Jiang *et al.*, 2022; Zuo *et al.*, 2019)
	QFN.nmbu_masbasis-7B.3	*AX-95235606*	(Jiang *et al.*, 2022; Mohan *et al.*, 2009)
	QFN.nmbu_masbasis-7B.4	*AX-158592462*	(Martinez *et al.*, 2018)
7D	*QFN.nmbu_masbasis-7D.1*	*AX-95155766*	(Cabral et al. 2014; Dhariwal et al. 2021; Jiang et al. 2022; Tai et al. 2021)
	QFN.nmbu_masbasis- 7D.2	*AX-95157119*	(Jaiswal et al. 2012; Jiang et al. 2022; Singh et al. 2010; Zhu et al. 2019; Zuo et al. 2019)
	QGI.nmbu_masbasis- 7D.3	*AX-94401880*	

Multivariate analysis

Explorative multivariate analysis by PCA across all genotypes from the trials n2, n6, n7 and n19 (Fig. 2A), reflect the dominating differences between adapted and non-adapted genotypes. PCA on only the adapted genotypes (Fig. 2B) separated a few genotypes from the others along the first component in the analysis of the adapted genotypes. Among these genotypes was the genotype T7347, previously shown to exhibit good PHS resistance (Buraas and

Skinnes 1985) and to carry important genes for PHS, as identified by transcriptome analyses (Begum 2023) and significant PHS-associated QTL (Ruud et al. 2025).

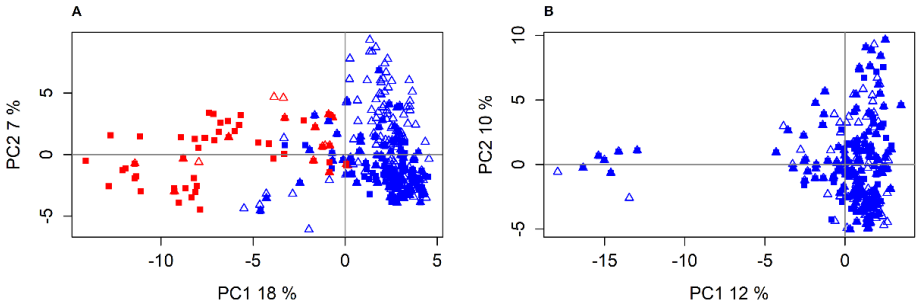


Fig. 2. PCA of MASBASIS trials of FN: n2 (89 genotypes), n6 (160 genotypes), n7 (287 genotypes) and n19 (299 genotypes), where 89 genotypes were present in all field trials. The data are one variable for each marker alleles, binarized as the presence versus absence of each alternative allele for each peak marker presented in Table 2, (A) PCA across all genotypes, displayed as score plot for adapted genotypes (blue) and non-adapted genotypes (red) (B) PCA applied only on the adapted genotypes (blue), displayed as score plot.

The supervised analyses, PLS, with the binarized markers as input and FN as response performed across the field trial with optimal PHS provocation (n2, n6, n7 and n19) are presented in Fig. 3. By PLS main pattern of the covariance structure between the genetic markers and FN are projected onto a few significant components. R2 of predicted vs observed FN was 0.47 in a model of all genotypes, 0.46 in a model of non-adapted genotypes, and 0.28 in a model of adapted genotypes at 3 components. The PLS loadings of the gene markers are numerically presentation for PLS of all genotypes in Table 3, and for the analyses among the non-adapted and among the adapted in Table S6. The frequencies of the alternative alleles are also presented. In PLS of FN across all genotypes, the adapted genotypes are located towards positive values along the first component. Thus, high values of the loadings of the first component are associated with high FN and PHS resistance. The markers with beneficial PHS resistance was also located positive along the first component in the analyses performed among the non-adapted genotypes and among the adapted genotypes.

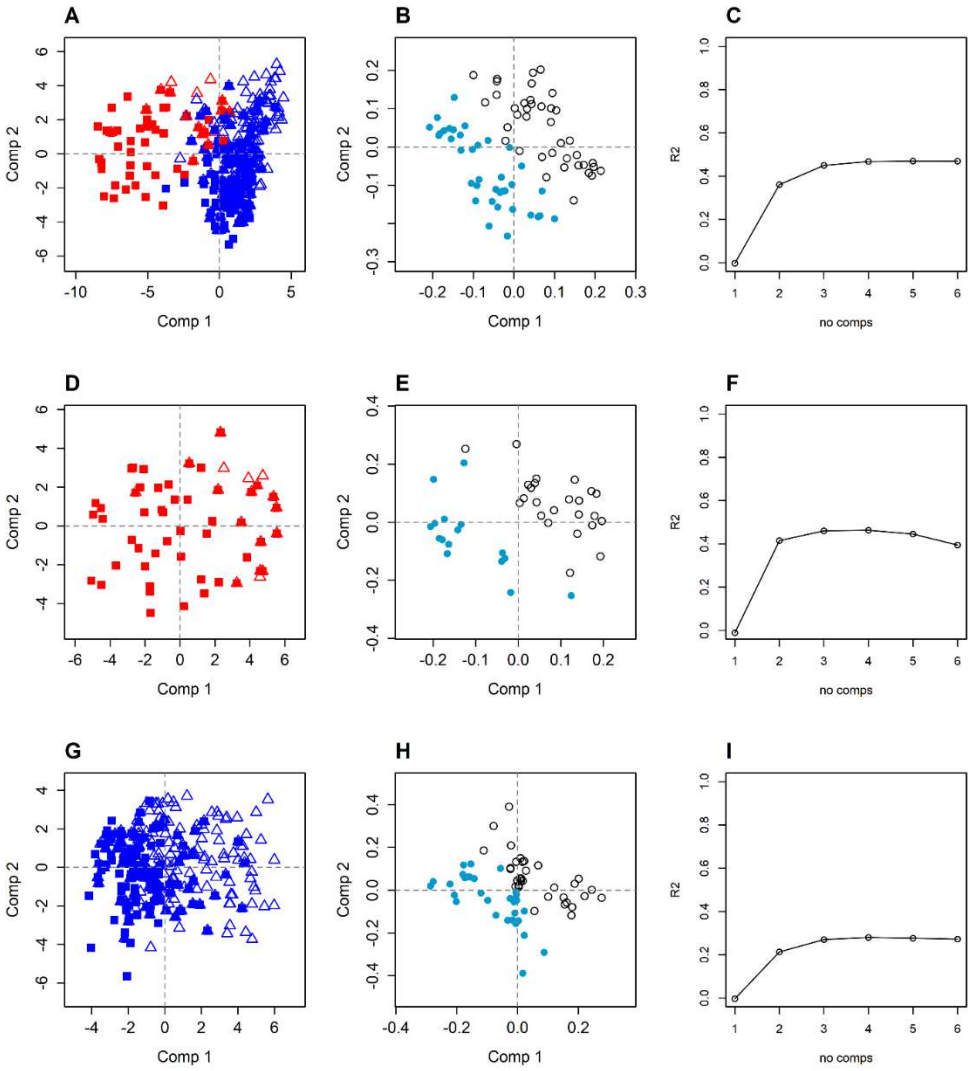


Fig. 3. PLS of MASBASIS of the FN trials: n2 (89 genotypes), n6 (160 genotypes), n7 (287 genotypes) and n19 (299 genotypes), where 89 genotypes were present in all field trials. The inputs are one variable for each marker alleles, binarized as the presence versus absence of each alternative allele for each peak marker presented in Table 2, and the response is FN. (A-C) all genotypes, (D-F) only non-adapted lines, and (G-I) only adapted lines. (A,D,G) 2D score plots of the samples for the two first and most important component for non-adapted (red) and adapted lines (blue) of genotypes. The symbols reflect mean FN within each trial, above FN mean (open triangles) and below mean FN (closed squares). (B,E,H) 2D loading plots of the genetic markers, positively (open black) and negatively (filled cyan) associated with FN. Numeric presentation of the loadings is provided in Table 3 and Table S6. (C,F,I). R2 of the PLS models with increasing component in the model along the x-axis.

The multivariate analysis applied to the trials with GI as response, including 4 field trials grown in Norway and 1 field trial grown in China (Fig. S8) resulted in R2 of 0.24 for the analysis of all data, 0.37 for the non-adapted genotypes, and 0.15 for the adapted genotypes. The loadings of the analyses across all genotypes are numerically

presentation in Table 3, and for the analyses among the non-adapted and among the adapted in Table S6. In PLS of GI across all data, low values across first component are associated with the beneficial low GI and high PHS resistance.

The PLS models across all genotypes reflect the generally higher FN and lower GI of the adapted genotypes compared with the non-adapted genotypes along. This is reflected along the first component, both in the analyses of FN and in the analyses of GI.

Many significant beneficial alleles had high frequencies among the adapted genotypes of MASBASIS and among the genotypes in the validation data, and relatively low frequencies among the non-adapted genotypes of MASBASIS (Table 3). One example is the marker *Tdurum_contig47083_278* (*QFN.nmbu_masbasis-1RS.1*). The beneficial allele of this marker is already present in most of the adapted genotypes. Another example is the marker allele, *AX-158558875_C* (*QFN.nmbu_masbasis-5A.2*), which had a frequency of 100% both in the adapted genotypes in MASBASIS and in the validation data, but only 43% in the non-adapted genotypes. In the analyses across all genotypes, these two marker alleles are positive loadings along the first component in the analyses of FN, thus positively associated with FN, and negatively loadings along the first component in the analysis of GI, thus associated with low GI, which both reflects beneficial effects on PHS resistance, and largely influenced by the differences between non-adapted and adapted genotypes.

Other markers with the same pattern, high loadings along the first component, are marker alleles *AX-158563556_G* (*QFN.nmbu_masbasis-3B.1*), *Kukri_c7436_2259_G* (*QFN.nmbu_masbasis-1A.3*), *AX-94735017_A* (*QFN.nmbu_masbasis-1A.1)*, *AX-94841408_T* (*QFN.nmbu_masbasis-7A.1*) and *AX-95157119_T* (*QFN.nmbu_masbasis-7D.2*), which are all present in more than 90% of the adapted genotypes in MASBASIS, and less frequent in the non-adapted material. These marker alleles were associated with high FN and low GI when analysed across all genotypes in MASBASIS.

Some of the beneficial allele markers had low frequency among the adapted genotypes in MASBASIS and the validation data, for example *AX-95187774_T* (*QFN.nmbu_masbasis-1A.2*) and *CAP12_c1816_325_C* (*QFN.nmbu_masbasis-7B.1*). The beneficial allele of these markers have high loading along the first component in the analyses of the adapted genotypes.

The two first components in a PLS analysis reflect two independent contributions to the responses. As example, in the analysis of FN among the adapted genotypes, in addition to the marker *AX-95187774*, the markers *tplb0045c05_547* and *AX-94420304* span the loadings along the first component, and *AX-158533842* is a marker which span the loadings along the second component. This shows a different pattern of variation for *AX-158533842* compared with the former, which may reflect a different underlying mechanism.

Allele stacking

Allele stacking was used to study the combined effects of multiple beneficial alleles. For allele stacking of MASBASIS, 34 beneficial alleles, based on the PLS loading and significance of the beneficial alleles, were included in the allele stacking (Table S7). A group variable was made as a sum of a binary variable for each beneficial allele (0 if absent and 1 if present), and a regression analyses with the group variable as input and FN or GI as response were performed. FN showed significant positive effect by combining multiple beneficial alleles up to a high number of beneficial alleles as analysed across all genotypes (Fig. 4). The results of allelic stacking are summarised in Fig 5a, which shows the mean FN by increasing number of beneficial alleles. Similar analyses performed among the non-adapted and among the adapted genotypes and summarised in Fig 5 b and c, respectively. Allelic stacking on GI by the same 34 beneficial alleles are summarised are presented in Fig 6.

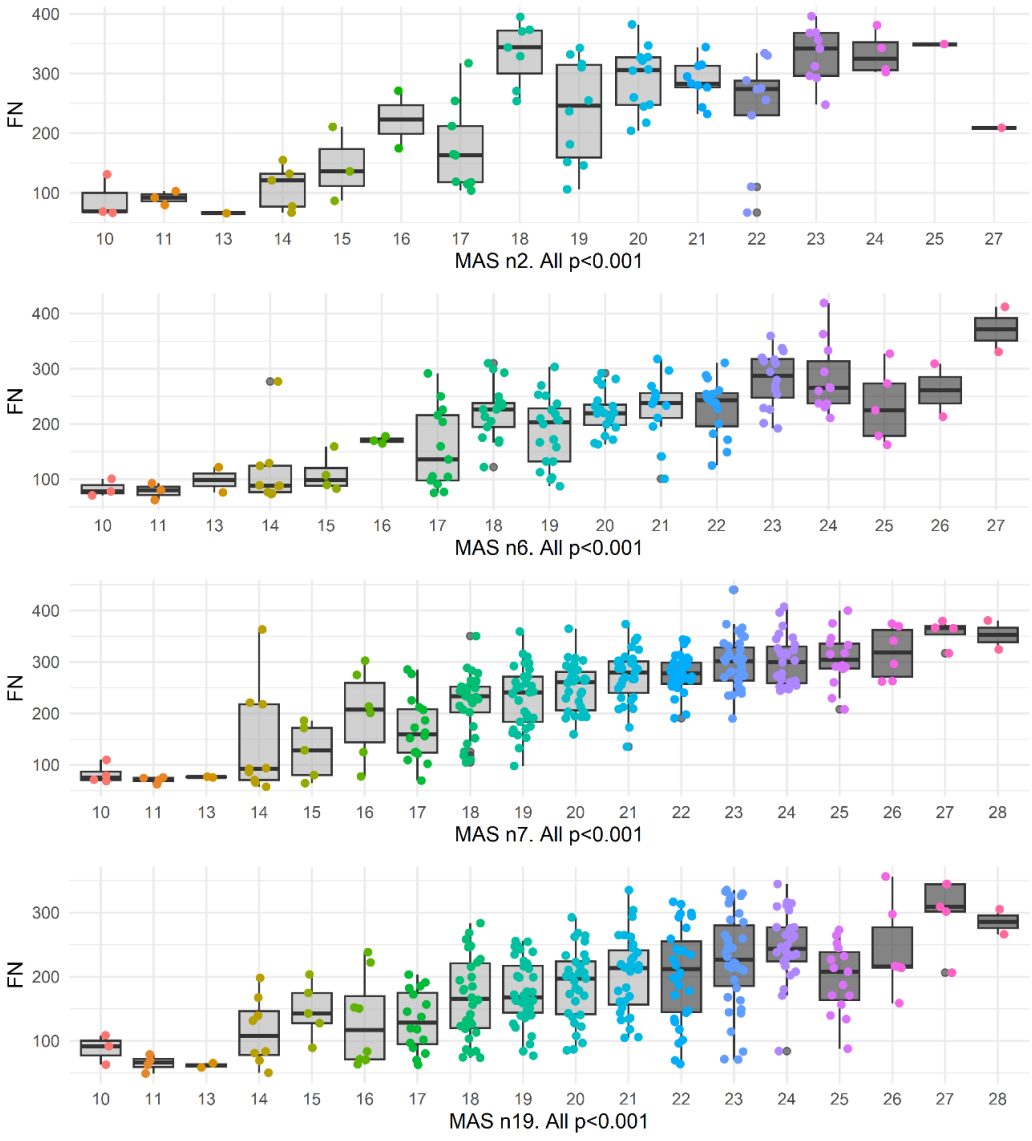


Fig. 4. Allelic stacking of MASBASIS all genotypes (All) of 34 significant markers from the trials: n2 (89 samples), n6 (160 samples), n7 (287 samples) and n19 (299 samples), where 89 genotypes were present in all field trials.

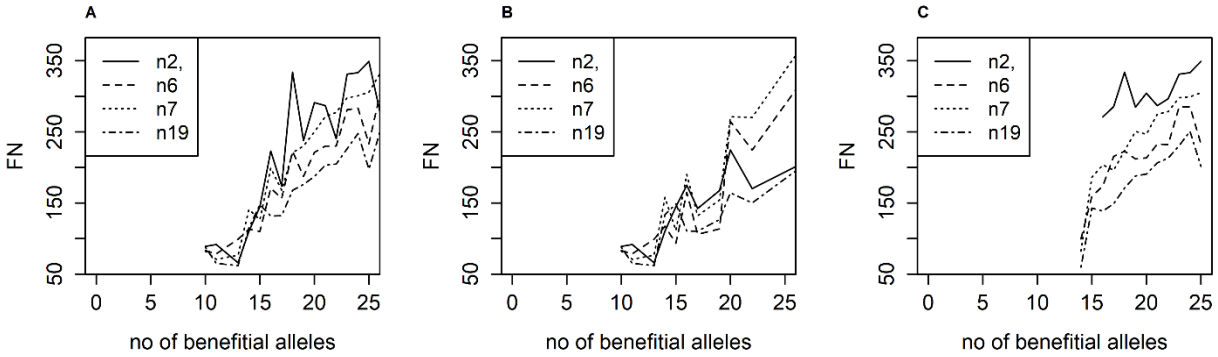


Fig 5. Mean FN of each experiment of (A) All genotypes, (B) non-adapted genotypes, and (C) adapted genotypes, plotted towards increasingly number of 34 beneficial alleles, selected from the PLS analyses of FN and GI. All models were significant ($p<0.001$) in the regression models with the number of alleles as group variable and the FN as response.

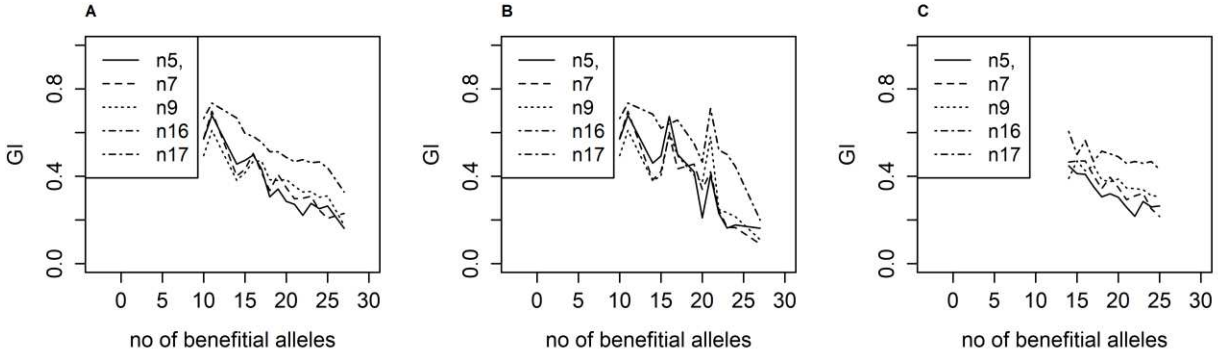
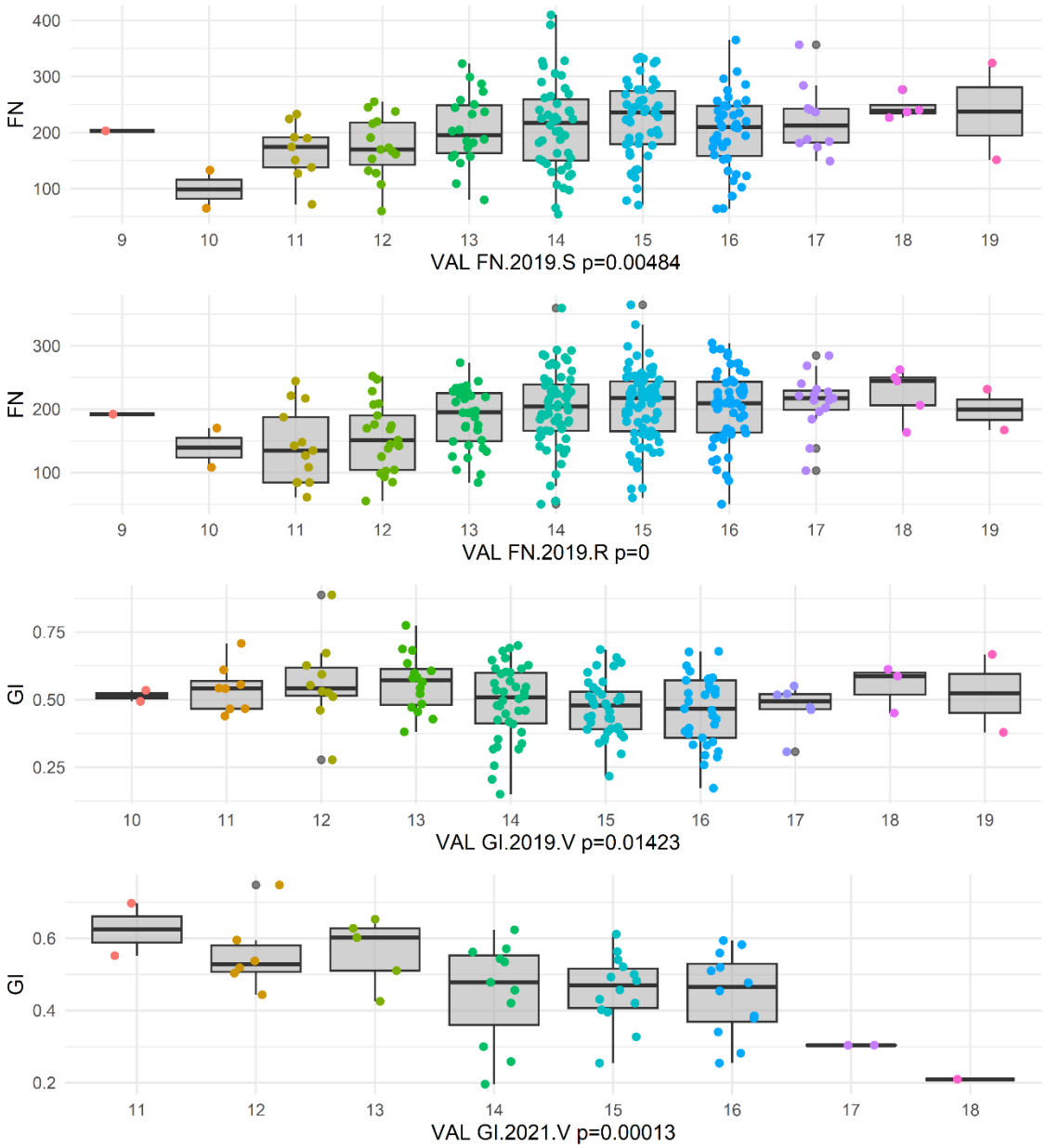


Fig 6. Mean GI of each experiment of (A) All genotypes, (B) non-adapted genotypes, and (C) adapted genotypes, plotted towards increasingly number of 34 beneficial alleles, selected from the PLS analyses of FN and GI. All models were significant ($p<0.001$) in the regression models with the number of alleles as group variable and the FN as response.

The mean FN and GI were improved with the number of the beneficial alleles included in all analyses. Among the non-adapted genotypes in MASBASIS, FN increased with the number of beneficial alleles from 10 up to 26. Many of these beneficial alleles, were, already implemented among the adapted material and the validation data. Among

441 the adapted genotypes in MASBASIS, most genotypes had 15 or more of the 37 tested beneficial alleles with a
442 gradual improvement of FN and GI up to 24-25 beneficial alleles.
443
444 Among the 34 beneficial alleles included in the MASBASIS, 23 were available in the validation data. In the
445 validation data, most genotypes had 11 of these 23 available beneficial alleles with improvements of a few more
446 beneficial alleles (Fig 7).
447



448
449

Fig. 7. Allelic stacking of the validation data (VAL) for FN and GI. Of the 37 significant markers included in the allele stacking of MASBASIS, 21 was available in the validation data. The trials were: n2 (89 samples), n6 (160 samples), n7 (287 samples) and n19 (299 samples), where 89 genotypes were present in all field trials.

Identification of relevant genes on the significant QTL by KnetMiner

Genes identified by KnetMiner located at a position range of +/- 2 Mbp of the peak markers in each significant QTL, filtered by relevance for PHS resistance, are presented in Table S8.

Some of the relevant genes, presented in Table S8 are presented here and commented in the discussion. The gene lipid phosphatase 2 (*LPP2*) at position 549.31 Mbp of chromosome 1A is in the in the range +/-2 Mbp of the marker *AX-95187774* (548.44-552.44 Mbp) in *QFN.nmbu_masbasis-1A.5*. The gene MOTHER of flowering locus T, terminal flower1 (*MFT* also called *PHS1*) at position 7.29 Mbp on chromosome 3A is in the range of the marker *AX-94993536* (4.69-8.69 Mbp) on (QFN.nmbu_masbasis-3A.1). The gene hydroxyethylthiazole kinase (*THIM*) at position 569.52 Mbp on 3D and late embryogenesis abundant hydroxyproline-rich glycoprotein family (*NHL39*) on 569.55 Mbp are in the range of the markers *AX-94791751* and *AX-95120609* (both at 569.23-573.23 Mbp), on *QGI.nmbu_masbasis-3D.1* and *QFN.nmbu_masbasis-3D.1*, respectively. The gene Plasma Membrane 19 family (*PM19L*) on position 604.07 Mbp on 4A are in the range of the marker BS00072025_51 (603.66-607.66 Mbp) on *QFN.nmbu_masbasis-4A.1/QGI.nmbu_masbasis-4A.1*. The gene acyl carrier protein 2, mitochondrial seed germination (*mtACP2*) on position *323.78* Mbp on 4B and the gene protein-serine/threonine phosphatase (*PP2C74*) at position 324.42 Mbp are in the range of *AX-94420304* (320.75-324.75 Mbp) on *QGI.nmbu_masbasis-4B.1*. The gene telomere repeat-binding protein 1 (*TRP1*) at position 662.36 Mbp on 5A are in the range of *AX-158584188* (662.27-666.27 Mbp) on *QGI.nmbu_masbasis-5A.1*. The gene peroxidase 44 (P44) on position 582.58 Mbp on 5B are in the range of the marker *AX-158533842* (578.69-582.69 Mbp) on *QFN.nmbu_masbasis-5B.1.* The gene γ-carbonic anhydrase-like 1, mitochondrial (*γ-CAL1*) at position 178.85 Mbp on 6D are in the range of *wsnp_Ex_c37749_45436366* (176.68-180.68 Mbp) on *QFN.nmbu_masbasis-6D.1*. The genes non-specific lipid-transfer protein 1 (*LP1*) at position 735.14 and ATP-binding cassette ABC transporters (*ABCG1*) at position 735.51 Mbp at 7A are in the range of the marker *tplb0045c05_547* (734.43-738.43) on *QFN.nmbu_masbasis-7A.2*, and sphingosine-1-phosphate *(S1P)* on position 22.06 Mbp are in the range of *CAP12_c1816_325* (19.67-23.67 Mbp) on *QFN.nmbu_masbasis-7B.1.*

Multivariate MAS modelling

As many QTL influences the PHS resistance, and they are here shown to have complementary effects, targeted MAS is challenging. We therefor propose a multivariate MAS selection where a multivariate model is made based on multiple markers to predict the phenotype. By selecting genotypes with high predicted phenotype (high FN and/or low GI), the frequency of beneficial alleles can be increased (Fig. 8). The composition of the beneficial alleles may be observed continuously to monitor the QTL of interest. Multivariate MAS models are suggested to be developed

on a training population on QTL that are validated both locally and from publications where their impact on PHS resistance is well funded. After well training of a multivariate MAS model on samples where both QTL and FN/GI are known, and the model is thereafter applied to predict the phenotype of new genotypes. Using PLS regression for multivariate MAS modelling gives a window to view the significant QTL and their correlation structure as demonstrated above in the PLS analyses.

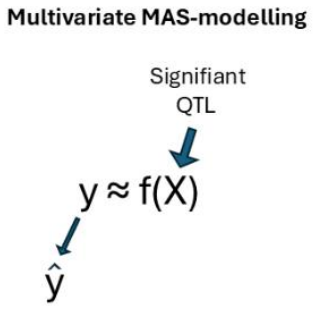


Fig. 8. Multivariate MAS-modelling applied.

Multivariate MAS can be combined with targeted MAS of particular QTL of interest, in a two-step procedure:

Step 1: Targeted MAS.
Reject genotypes according to some crucial genes/markers.
For Norwegian condition: ensure at least one red *R-1* gene.
Consider other QTL of particular interest.

Step 2: Multivariate MAS.
Make a multivariate model for significant QTL based on a training set and apply the model on new genotypes to select genotypes with increasing frequency of beneficial alleles.
Validate the results by monitoring the frequencies of the relevant beneficial alleles and the phenotype.

Discussion

The provocation of PHS varies from year to year due to seasonal variation, which challenges the use of FN as a selection criterion for PHS resistance, and GI is time and labour consuming. Therefore, reliable QTL are needed for breeding wheat for improved dormancy and PHS resistance. In view of speed breeding, the need for efficient selection tool for PHS resistance is urgently needed.

MASBASIS, covering both adapted and non-adapted genotypes, is here tested in many different field trials. Due to variable exposure to PHS provocation in the field and mist irrigation in some field trials, large variations in the level of FN and GI were observed. Many markers, distributed throughout the genome, were significant for FN and GI by GWAS, which was performed across all genotypes, and thus represent both international relevant genotypes and

adapted genotypes. The use of both GI, which reflect primary dormancy, and FN, which reflect also the exposure to PHS provocation during maturation, have captured different properties relevant for PHS resistance. As GWAS was applied both on the individual field trial, and on three groups defined by mean FN as high, medium and low FN, a large span of PHS provocation are considered by the GWAS. All the significant QTL are aligned with published QTL using a 30 Mbp threshold for overlap. References to the publications for each QTL are presented in Table 2B, and a few are commented on in this discussion.

The multivariate analyses, which were applied only on field trials with the most optimal variation in FN for PHS resistance and for the GI field trials, have enabled identification of markers that are most relevant among the adapted markers and among the non-adapted genotypes. A limitation is that these analyses were performed after selection by GWAS across all genotypes, which means that the results of GWAS are strongly influenced by the differences between adapted and non-adapted genotypes, and there may be other QTL among the adapted genotypes that are not identified in this publication. Yet, numerous QTL were observed as significant both across all data, among the non-adapted genotypes and among the adapted genotypes. Several QTL were also significant in local external validation data, although not all QTL could be tested as two SNP ships were applied in MASBASIS and only one of them were applied in the validation data set.

The significant QTL are distributed throughout the chromosomes, and importantly, they show complementary effect up to a high number of QTL as verified by allele stacking. For each significant QTL, KnetMiner identified several genes that code for proteins with relevance to PHS. KnetMiner uses IWGSC RefSeq v1.0 for wheat, loaded via Ensembl Plants to identify genes, and keywords are added as filter in the analyses to focus on the genes that have relevance for PHS resistance. All genes identified by KnetMiner are presented in Table S8, and a few of these genes are commented on in this discussion. The identified genes are within the range of +/- 2Mbp on each side of the markers, which imply that they are located well within the respective QTL. The observation that the identified genes code for proteins with fundamentally different molecular function supports the conclusion that many of the identified QTL have complementary impact on PHS resistance.

The marker *Tdurum_contig47083_278* (*QFN.nmbu_masbasis-1RS*), which showed significant effect on FN by the multivariate analysis when analysed across all genotypes and among the non-adapted genotypes, is already implemented in the adapted genotypes. *Tdurum_contig47083_278* was previously detected in the biparental population SHA3/CBRD × Naxos (Begum et al. 2025). It was found that the physical position of this marker corresponds to QTL for the well-known wheat/rye 1BL.1RS translocation in wheat (Rabinovich 1998). Whereas this translocation is widely used for improved yield in many countries (Zhao et al. 2012), it is avoided in the Norwegian wheat breeding due to its negative effects on the bread wheat quality. Based on the well-known effect of the 1BL.1RS translocation on PHS it is likely that this gene is one of the reasons for dominating higher FN in adapted than non-adapted genotypes.

In addition, there were several significant QTL which also had high abundance (90-100%) of the beneficial alleles in the adapted material, and lower in the non-adapted material. Examples are the marker alleles *AX-158563556_G* (*QFN.nmbu_masbasis-3B.1*), *Kukri_c7436_2259_G* (*QFN.nmbu_masbasis-1A.3*), *AX-94735017_A* (*QFN.nmbu_masbasis-1A.1)*, *AX-94841408_T* (*QFN.nmbu_masbasis-7A.1*) and *AX-95157119_T* (*QFN.nmbu_masbasis-7D.2*).

Some beneficial QTL alleles were low both among the adapted and the non-adapted genotypes. One example is the marker *AX-94420304* (*QFN.nmbu_masbasis-4B.1*) located at the position 322.75 Mbp which showed the highest number of fields with significant results in GWAS, and the beneficial allele was low in all data sets. In the multivariate analyses of FN this marker had the high loading along the first and most important component. One of the gene identified on *QFN.nmbu_masbasis-4B.1*, as observed by KnetMiner is *mtACP2*, which is a key component of mitochondrial fatty acid biosynthesis, required for physiological metabolism (Fu et al. 2020). Another gene on this QTL is the protein phosphatase *PP2C74*, which belongs to one of the largest families identified in plants (Xue et al. 2008). Phosphatase 2C is found to be a regulator of ABA responses (Gosti et al. 1999). This marker was not available in the validation data.

The marker *tplb0045c05_547* on *QFN.nmbu_masbasis-7A.2* displayed a similar pattern as *AX-94420304* in the multivariate analyses across all data and among the adapted genotypes. One gene in the range of this marker is the non-specific lipid-transfer protein *(LP1)*, a key processes of plant cytology, such as the stabilization of membranes, cell wall organization, and signal transduction (Liu et al. 2015a), another gene is the ATP-binding cassette ABC transporters (*ABCG1*) known to play a role in phytohormone transport (Do et al. 2018).

The marker *CAP12_c1816_325* (*QFN.nmbu_masbasis-7B.1*) is a significant marker with low frequency of the beneficial allele both among the non-adapted and among the adapted genotypes. The favourable allele of this QTL is present in 57% of the non-adapted and 20% of the adapted genotypes of MASBASIS, and in 6% of the lines in the validation data. Positive association with PHS within the corresponding physical region for this QTL is reported (Rabieyan et al. 2022) associated with germination percentage, sprouting index, sprouting score, and α-amylase. KnetMiner identified the gene *S1P* as potentially relevant gene on this marker. *S1P* is an important signalling molecule (Worrall et al. 2008).

The marker *AX-95187774* (*QFN.nmbu_masbasis-1A.5*) was significant for FN in the multivariate analyses of the adapted genotypes. Among the genes identified by KnetMiner on this marker is lipid phosphatase 2 (*LPP2*). *LPP2* is involved in lipid signaling in plants by conversion of phosphatidic acid to diacylglycerol, and it is found to play a role in ABA signalling (Katagiri et al. 2005).

The marker *AX-158533842* (*QFN.nmbu_masbasis-5B.1*), observed to have the highest loading along the second component in PLS of the adapted genotypes. One of the gene identified on this QTL is peroxidase 44 (*P44)*, which

break down hydrogen peroxide (H_2O_2), a major metabolite in redox sensing, signalling and regulation (Amir et al. 2026; Bailly et al. 2008; Singh et al. 2015). The frequency of the beneficial allele of *QFN.nmbu_masbasis-5B.1* was 44 % for the non-adapted material in MASBASIS, 36 % for the adapted genotypes in MASBASIS, and 69 % in the local validation data.

Another QTL, significant for FN in the adapted material with high loading along second component in the analysis of FN is the marker *wsnp_Ex_c37749_45436366* (*QFN.nmbu_masbasis-6D.1*), located at 178.68 Mbp. The beneficial allele of this marker is present in 61% and 66% of the non-adapted and adapted material of MASBASIS, respectively, and 78% in the validation data. KnetMiner identified several candidate genes for this marker, including gamma carbonic anhydrase-like 1, mitochondrial (*γ-CAL1*), which belong to the γ-CA domain, essential for complex I assembly, mitochondrial potential, embryo development and seed germination (Cordoba et al. 2016; Ghifari et al. 2023).

AX-94791751 and *AX-95120609* (on *QGI.nmbu.masbasis 3D.1* and *QFN.nmbu_masbasis-3D.1*, respectively), located 571.23 Mbp, also showed a similar pattern to *wsnp_Ex_c37749_45436366* (*QFN.nmbu_masbasis-6D.1*) and *AX-158533842* (*QFN.nmbu_masbasis-5B.1*) in the multivariate analyses of FN for the adapted genotypes: low along the first component, and high along the second component in the analyses of FN. In the analyses of GI, these QTL had high dominating loadings along both components of the adapted material. The beneficial allele of the QTL on 3D had a relatively low frequency, 26-30%, both among all datasets in MASBASIS. KnetMiner identified three putative genes as significant for PHS. Among the genes was hydroxyethylthiazole kinase (*THIM*), which is involved in thiamine salvage pathway, an essential cofactor in the metabolism (Yazdani et al. 2013). Another gene was the late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family, *NHL39*. Hao and coworkers (Hao et al. 2025) described that LEA expression is fine-tuned by a multi-layered regulatory network that integrates environmental signals with intracellular second messengers by Ca^{2+} and kinase cascades (MAPK), with the ABA signaling pathway acting as the central backbone for this extensive crosstalk.

The QTL *QFN.nmbu_masbasis-3A.1* comprising the two markers *BS00092728_51* and *AX-94993536* located on the short arm of 3A had high loadings on the second component both in the analyses of FN and in the analyses of GI. The beneficial allele of the marker *BS00092728_51* is frequent in all data: 96% of the non-adapted genotypes, 84 % of the adapted genotypes, and 91% in the local validation data. KnetMiner showed that this QTL include several potentially relevant genes. One of the genes is *TaPHS1/TaMFT* located at the position 7.25 Mbp (Nakamura et al., 2011), which is well known to be associated with PHS resistance (Cao et al., 2016; Dhariwal et al., 2021; Himi et al., 2024; Lin et al., 2016; Liu and Bai, 2010; Liu et al., 2008; Liu et al., 2013; Liu et al., 2015b; Martinez et al., 2018; Mohan et al., 2009; Mori et al., 2005; Moullet et al., 2022; Nakamura et al., 2011; Osa et al., 2003; Shao et al., 2018; Zhu et al., 2019; Zuo et al., 2019).

The marker *BS00072025_51* (*QFN.nmbu_masbasis-4A.1/QGI.nmbu_masbasis-4A.1*) was significant for GI in GWAS as analysed across all GI experiments. This QTL has previously been found to be associated with PHS resistance (Barrero et al. 2015; Begum et al. 2025; Dallinger et al. 2024; Zhu et al. 2019) and overlaps with the *Phs1* locus on 4AL at 604 Mbp (Barrero *et al.*, 2015; Shorinola et al., 2016). The favourable allele of the marker *BS00072025_51*, located at 605.66 Mbp, was present in 69% of the non-adapted genotypes, 90% of the adapted material of MASBASIS, and 98 % of the Norwegian breeding population. By KnetMiner this QTL covers several PHS associated genes, including the membrane protein *PM19L*. *PM19-A1* is closely linked to *TaMKK3-A*, located within a 1.2 Mb physical interval. Genetic characterization of diverse germplasm showed that *TaMKK3-A*, and not *PM19*, is the major gene underlying the *Phs-A1* effect in European, North American, Australian and Asian germplasm (Shorinola et al. 2017). The beneficial allele of this marker was more frequent in the adapted than the non-adapted material: 69% among the non-adapted genotypes and 90% among the adapted genotypes in MASBASIS, and 98% in the validation data.

The marker *AX-95157119* (*QFN.nmbu_masbasis-7D.2*) located at 585.15 Mbp, with a frequency of 95% for the beneficial allele in the adapted material of MASBASIS, and 42% among the non-adapted genotypes, corresponds to a QTL previously described for PHS resistance Jaiswal et al. (2012) and for field sprouting, GI, and period of dormancy within the same chromosome region on 7DL (Zhu et al. 2019). This QTL is already strongly implemented in the Norwegian breeding lines, as reflected by a frequency of 0.98 in the local validation data.

The beneficial allele of the marker *AX-158558875* (*QFN.nmbu_masbasis-5A.2*) located at 535.75 Mbp, was present in all adapted genotypes in MASBASIS and in all genotypes in the validation data, but only 43% among the non-adapted genotypes in MASBASIS. This QTL corresponds to a previously described QTL region (Dhariwal et al. 2021) and (Martinez et al. 2018).

The highest loading along the second PLS component in the multivariate analyses of GI across all genotypes and across the adapted genotypes was *AX-158584188_G* (*QGI.nmbu_masbasis-5A.1*). One of the putative gene observed by KnetMiner on this QTL is the telomere repeat-binding protein 1 (*TRP1*). Changes in telomeres can affect whole-genome stability (Peska and Garcia 2020).

Another marker with high loadings along the first component in the analysis of GI is *AX-94505411_C* (*QGI.nmbu_masbasis_7B.1*). This marker was only significant for GI and not for FN. One of the genes identified on this QTL is *AOX1A*. The AOX protein plays a crucial role in low oxygen metabolism because it replaces oxygen by nitrite as terminal electron acceptor in the electron transport chain (Jayawardhane et al. 2020). Oxygen is a limiting factor in wheat germination (Alani et al. 1985), and during early seed germination before the seed coat is broken. Oxidative pathways at this stage cannot operate with physiologically significant intensity due to limitations in oxygen availability (Bykova and Igamberdiev 2025). The beneficial allele of *AX-94505411* was present in only 14% of the non-adapted genotypes, and 65% among the adapted genotypes, whereas it was not available for the validation data set.

Our results on allelic stacking showed that multiple QTL have supplementary impact on FN and GI. This observation is supported by the observation that the QTL were identified by KnetMiner to contain genes with fundamentally different molecular functions. Although it is not known which genes are causal for the identified genetic marker, the observation that many genes with fundamentally different functions are located in the range of these QTL, support the observations from allelic stacking that multiple markers display complementary effects in contributing to stable PHS resistance. Many significant QTL have high frequency of beneficial alleles in the Norwegian breeding population as result of decades of selection for PHS resistance. This observation provides real-world validation of the identified QTL, and it implies honour to the long-term breeding of Norwegian wheat, which has successfully incorporated many beneficial genes for PHS resistance through selection for high FN in the field over decades. There is, however, a need to improve further the PHS resistance of wheat grown in Norway, and several candidate QTL with positive complementary effects on PHS are identified in the adapted material in this study.

The observation that a high number of QTL for PHS resistance is needed to avoid field sprouting, imply challenges for wheat breeding. In addition to PHS sprouting, many other phenotypic traits must also be considered. As many significant QTL contribute complementary to PHS resistance, we suggest multivariate MAS modelling to improve PHS resistance by plant breeding, which enable many QTL to be considered simultaneously to increase the frequency of beneficial alleles, while maintaining beneficial alleles, and to providing transparent visualisation of the QTL and their correlation structures. The observation that the identified QTL are also aligned with published QTL and cover genes with relevance to PHS sprouting, gives validity of the identified QTL, and it supports a strategy of multivariate MAS for selection of PHS resistance. The multivariate supervised method PLS is suggested as it utilize the correlation structures and provides visual plots of the main pattern that may be utilised. Multivariate MAS can be combined with targeted MAS of a few specific markers of interest.

The use of high of speed wheat breeding, where the off-season is utilized, makes multivariate MAS even more relevant. In breeding for PHS resistance, it is not only important to incorporate new genes for PHS resistance, but also to maintain the good genes for PHS resistance. It is therefore important to consider many genetic markers for PHS resistance.

In conclusion, our results show that although breaking dormancy and the initiation of germination are strictly coordinated, there are many genetic factors that influence this process, and a genotype must have beneficial alleles of many different genes for a suitable PHS resistance. In plant breeding of improved PHS resistance, a few important QTL with well-known effect may be applied by targeted MAS to maintain/improve their high frequency, such as QTL for at least one of the known major red colour genes that are needed under Norwegian condition, whereas a multivariate MAS is suggested to increase the frequency of other beneficial alleles and simultaneously display insight into the correlation structure of these QTL in the data.

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Author Contributions

MCB, ML and AKU planned the research. ML performed the material preparation and experimental design, data handling, commented, and contributed to the discussions. MCB performed field recording data from Vollebekk field trials, GI analysis, data handling, GWAS analysis and validation, multivariate analysis, and prepared the first draft manuscript. AKU contributed to the field recording, GI analysis, data handling, and the discussion. AKR contributed to the discussions. JAD was responsible for field trials and recordings in Staur and measuring FN. EMF supervised the multivariate data analyses, contributed to the manuscript and the discussion, and is responsible for the submission of the manuscript. EY was responsible for conducting the field trials, recording data, and performing GI analysis from Chengdu field trials. All authors have read and approved the final manuscript.

Ethical standards

The experiments conducted in this study comply with the current laws of Norway.

Data availability

Data of all genetic markers identified in MASBASIS are available in:

https://nofima.no/wp-content/uploads/2025/12/MASBASIS-genotype-data.-35K-For-Data-Availability-Statement.pdf

https://nofima.no/wp-content/uploads/2025/12/MASBASIS-genotype-data.-25K.-For-Data-Availability-Statement.pdf

A breeding population from the Norwegian Breeding population was used for independent validation of the genetic markers that were significant in MASBASIS. Data on the phenotype and the significant markers presented in Table 2 are provided for the validation data in Table S4.

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Declaration of interests

The authors declare that they have no conflicts of interest.

References

Alani A, Bruzau F, Raymond P, Saintges V, Leblanc JM, Pradet A (1985) Germination, Respiration, and Adenylate Energy-Charge of Seeds at Various Oxygen Partial Pressures. Plant Physiology 79:885–890

Albrecht T, Oberforster M, Kempf H, Ramgraber L, Schacht J, Kazman E, Zechner E, Neumayer A, Hartl L, Mohler V (2015) Genome-wide association mapping of preharvest sprouting resistance in a diversity panel of European winter wheats. J Appl Genet 56:277–285

Amir M, Sandhu R, Khan FA, Moinuddin, Ahmad B (2026) Oxidative Signalling in Seed Dormancy Release and Germination: The Role of Reactive Oxygen Species. J Plant Growth Regul

Arif MAR, Neumann K, Nagel M, Kobiljski B, Lohwasser U, Börner A (2012) An association mapping analysis of dormancy and pre-harvest sprouting in wheat. Euphytica 188:409–417

Bailly C, El-Maarouf-Bouteau H, Corbineau F (2008) From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. Cr Biol 331:806–814

Barrero JM, Cavanagh C, Verbyla KL, Tibbits JFG, Verbyla AP, Huang BE, Rosewarne GM, Stephen S, Wang PH, Whan A, Rigault P, Hayden MJ, Gubler F (2015) Transcriptomic analysis of wheat near-isogenic lines identifies PM19-A1 and A2 as candidates for a major dormancy QTL. Genome Biology 16:1–18

Batista LA, Badillo N, Friskop A, Green A (2024) Accelerating genetic gain through strategic speed breeding in spring wheat. Crop Sci 64:3311–3322

Begum MC (2023) The Genetic and Molecular Basis for Pre-Harvest Sprouting Resistance in Spring Wheat adapted to Norwegian Growing Condition. Faculty of Biosciences, Department of Plant Science. Norwegian University of Life Sciences, p 303

Begum MC, Belova T, Dieseth JA, Yang EN, Mosleth EF, Uhlen AK, Lillemo M (2025) QTL mapping of preharvest sprouting resistance in bread wheat population SHA3/CBRD by Naxos confirms a negative effect of the 1BL.1RS translocation on falling number. Crop Sci 65

Bewley JD (1997a) Seed germination and dormancy. Plant Cell 9:1055–1066

Bewley JD (1997b) Seed Germination and Dormancy. The Plant Cell 9:1055–1066

Bradford KJ, Trewavas AJ (1994) Sensitivity Thresholds and Variable Time Scales in Plant Hormone Action. Plant Physiology 105:1029–1036

Buraas T, Skinnes H (1985) Development of Seed Dormancy in Barley, Wheat and Triticale under Controlled Conditions. Acta Agr Scand 35:233–244

Bykova NV, Igamberdiev AU (2025) Redox Control of Seed Germination is Mediated by the Crosstalk of Nitric Oxide and Reactive Oxygen Species. Antioxid Redox Sign 42:442–461

Börner A, Nagel M, Agacka-Moldoch M, Gierke PU, Oberforster M, Albrecht T, Mohler V (2018) QTL analysis of falling number and seed longevity in wheat (Triticum aestivum L.). J Appl Genet 59:35–42

Cabral AL, Jordan MC, McCartney CA, You FM, Humphreys DG, MacLachlan R, Pozniak CJ (2014) Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (Triticum aestivum L.). Bmc Plant Biol 14

Cao LZ, Hayashi K, Tokui M, Mori M, Miura H, Onishi K (2016) Detection of QTLs for traits associated with pre-harvest sprouting resistance in bread wheat (Triticum aestivum L.). Breeding Sci 66:260–270

Chang C, Feng JM, Si HQ, Yin B, Zhang HP, Ma CX (2010a) Validating a novel allele of viviparous-1 (Vp-1Bf) associated with high seed dormancy of Chinese wheat landrace, Wanxianbaimaizi. Mol Breeding 25:517–525

Chang C, Zhang HP, Feng JM, Yin B, Si HQ, Ma CX (2010b) Identifying alleles of Viviparous-1B associated with pre-harvest sprouting in micro-core collections of Chinese wheat germplasm. Mol Breeding 25:481–490

Chao SM, Xu SS, Elias EM, Faris JD, Sorrells ME (2010) Identification of Chromosome Locations of Genes Affecting Preharvest Sprouting and Seed Dormancy Using Chromosome Substitution Lines in Tetraploid Wheat (*Triticum turgidum L*.). Crop Sci 50:1180–1187

Chen CX, Cai SB, Bai GH (2008) A major QTL controlling seed dormancy and pre-harvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. Mol Breeding 21:351–358

Chen L, Tao Y, Song CX, Liu YK, Tong HW, Ning Q, Zou J, Fu PH, Zhang YQ, Gao CB, Zhu ZW (2025) Genome-wide association study of pre-harvest sprouting resistance and grain color in common wheat (Triticum aestivum L.). Bmc Plant Biol 25

Cheng MJ, Guo YL, Liu Q, Nan SW, Xue YX, Wei CH, Zhang Y, Luan FS, Zhang X, Li H (2022) H2O2 and Ca2+ Signaling Crosstalk Counteracts ABA to Induce Seed Germination. Antioxidants-Basel 11:1–12

Cordoba JP, Marchetti F, Soto D, Martin MV, Pagnussat GC, Zabaleta E (2016) The CA domain of the respiratory complex I is required for normal embryogenesis in. J Exp Bot 67:1589–1603

Dallinger HG, Löschenberger F, Azrak N, Ametz C, Michel S, Bürstmayr H (2024) Genome-wide association mapping for pre-harvest sprouting in European winter wheat detects novel resistance QTL, pleiotropic effects, and structural variation in multiple genomes. Plant Genome-Us 17

Dhariwal R, Hiebert CW, Sorrells ME, Spaner D, Graf RJ, Singh J, Randhawa HS (2021) Mapping pre-harvest sprouting resistance loci in AAC Innova x AAC Tenacious spring wheat population. Bmc Genomics 22

Ditz N, Brandt D, Gasper M, Röhricht H, Hegermann J, Wallbott A, Eirich J, Blum N, Rugen N, Niehaus M, del Pilar Martinez M, Heinemann B, Kühn K, Maurino VG, Müller-Schüssele S, Hildebrandt TM, Schwarzländer M, Braun H-P, Herde M, Meyer EH, Eubel H, Finkemeier I (2026) Seed mitochondria are equipped with cristae and a full proteome to kickstart germination. Curr Biol 36:4320–4339

Do THT, Martinoia E, Lee Y (2018) Functions of ABC transporters in plant growth and development. Curr Opin Plant Biol 41:32–38

Farrell AD, Kettlewell PS, Simmonds J, Flintham JE, Snape JW, Werner P, Jack PL (2013) Control of late maturity alpha-amylase in wheat by the dwarfing gene Rht-D1b and genes on the 1B/1R translocation. Mol Breeding 32:425–436

Fofana B, Humphreys DG, Rasul G, Cloutier S, Brûlé-Babel A, Woods S, Lukow OM, Somers DJ (2009) Mapping quantitative trait loci controlling pre-harvest sprouting resistance in a red x white seeded spring wheat cross. Euphytica 165:509–521

Fu XY, Guan X, Garlock R, Nikolau BJ (2020) Mitochondrial Fatty Acid Synthase Utilizes Multiple Acyl Carrier Protein Isoforms. Plant Physiology 183:547–557

Ghifari AS, Saha S, Murcha MW (2023) The biogenesis and regulation of the plant oxidative phosphorylation system. Plant Physiology 192:728–747

Gosti F, Beaudoin N, Serizet C, Webb AAR, Vartanian N, Giraudat J (1999) ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. Plant Cell 11:1897–1909

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet D (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. Theoretical and Applied Genetics 104:39–47

Hagberg S (1961) Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem 38:202–+

Hanft JM, Wych RD (1982) Visual Indicators of Physiological Maturity of Hard Red Spring Wheat. Crop Sci 22:584–588

Hao CH, Zhan XX, Guo N, Liu J, Cui DY (2025) LEA proteins and ABA signaling: reciprocal regulation in stress adaptation. Front Plant Sci 16

Himi E, Kurihara-Yonemoto S, Abe F, Takahashi H, Tanaka K, Matsuura T, Maekawa M, Sasaki T, Rikiishi K (2024a) Tamby10-D1 restores red grain color and increases grain dormancy via suppressing expression of TaLP2.128, non-specific lipid transfer protein in wheat. Euphytica 220

Himi E, Kurihara-Yonemoto S, Abe F, Takahashi H, Tanaka K, Matsuura T, Maekawa M, Sasaki T, Rikiishi K (2024b) Tamyb10-D1 restores red grain color and increases grain dormancy via suppressing expression of TaLTP2.128, non-specific lipid transfer protein in wheat. Euphytica 220

Himi E, Maekawa M, Miura H, Noda K (2011) Development of PCR markers for Tamyb10 related to R-1, red grain color gene in wheat. Theoretical and Applied Genetics 122:1561–1576

Hull SI, Swanepoel PA, Botes WC (2024) A critical review of the factors influencing pre-harvest sprouting of wheat. Agron J 116:3354–3367

Jaiswal V, Mir RR, Mohan A, Balyan HS, Gupta PK (2012) Association mapping for pre-harvest sprouting tolerance in common wheat (Triticum aestivum L.). Euphytica 188:89–102

Jayawardhane J, Cochrane DW, Vyas P, Bykova N, Vanlerberghe GC, Igamberdiev AU (2020) Roles for Plant Mitochondrial Alternative Oxidase Under Normoxia, Hypoxia, and Reoxygenation Conditions. Front Plant Sci 11

Jia Y, Barrero JM, Wang JR, Considine MJ, Nakamura S, Li CD (2024) Seed dormancy, germination, and pre-harvest sprouting, volume II. Front Plant Sci 15

Jiang H, Fang Y, Yan D, Liu ST, Wei J, Guo FL, Wu XT, Cao H, Yin CB, Lu F, Gao LF, Liu YX (2022) Genome-wide association study reveals a NAC transcription factor TaNAC074 linked to pre-harvest sprouting tolerance in wheat. Theoretical and Applied Genetics 135:3265–3276

Jiang H, Gao W, Jiang BL, Liu X, Jiang YT, Zhang LT, Zhang Y, Yan SN, Cao JJ, Lu J, Ma CX, Chang C, Zhang HP (2023) Identification and validation of coding and non-coding RNAs involved in high-temperature-mediated seed dormancy in common wheat. Front Plant Sci 14

Katagiri T, Ishiyama K, Kato T, Tabata S, Kobayashi M, Shinozaki K (2005) An important role of phosphatidic acid in ABA signaling during germination in Arabidopsis thaliana. Plant J 43:107–117

Kulwal P, Ishikawa G, Benscher D, Feng ZY, Yu LX, Jadhav A, Mehetre S, Sorrells ME (2012) Association mapping for pre-harvest sprouting resistance in white winter wheat. Theoretical and Applied Genetics 125:793–805

Kumar A, Kumar J, Singh R, Garg T, Chhuneja P, Balyan HS, Gupta PK (2009) QTL analysis for grain colour and pre-harvest sprouting in bread wheat. Plant Sci 177:114–122

Kumar S, Knox RE, Clarke FR, Pozniak CJ, DePauw RM, Cuthbert RD, Fox S (2015) Maximizing the identification of QTL for pre-harvest sprouting resistance using seed dormancy measures in a white-grained hexaploid wheat population. Euphytica 205:287–309

Lang J, Fu YX, Zhou Y, Cheng MP, Deng M, Li ML, Zhu TT, Yang J, Guo XJ, Gui LX, Li LC, Chen ZX, Yi YJ, Zhang LQ, Hao M, Huang L, Tan C, Chen GY, Jiang QT, Qi PF, Pu ZE, Ma J, Liu ZH, Liu YJ, Luo MC, Wei YM, Zheng YL, Wu YR, Liu DC, Wang JR (2021) MyB10-D confers PHS-3D resistance to pre-harvest sprouting by regulating NCED in ABA biosynthesis pathway of wheat. New Phytol 230:1940–1952

Lewsey MG, Bassel GW, Whelan J (2025) Dynamic and spatial control of cellular activity during seed germination. Curr Opin Plant Biol 86

Li RQ, Hameed MU, Geuten K (2026) Translating research on seed dormancy and germination from Arabidopsis to temperate cereals to control pre-harvest sprouting. Front Plant Sci 17
Li Z, Chen YY, Ou X, Wang MN, Wang NX, Li W, Deng YW, Diao YX, Sun ZX, Luo QY, Li XL, Zhao LQ, Yan T, Peng WH, Jiang Q, Fang Y, Ren ZL, Tan FQ, Luo PG, Ren TH (2022) Identification of a stable major-effect quantitative trait locus for pre-harvest sprouting in common wheat (Triticum aestivum L.) via high-density SNP-based genotyping. Theoretical and Applied Genetics 135:4183–4195
Lin M, Cai SB, Wang S, Liu SB, Zhang GR, Bai GH (2015) Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. Theoretical and Applied Genetics 128:1385–1395
Lin M, Dieseth JA, Alsheikh M, Yang EN, Holzapfel J, Schuermann F, Morales L, Michel S, Buerstmayr H, Bhavani S, Lillemo M (2023) A major yellow rust resistance QTL on chromosome 6A shows increased frequency in recent Norwegian spring wheat cultivars and breeding lines. Theoretical and Applied Genetics 136
Lin M, Ficke A, Dieseth JA, Lillemo M (2022) Genome-wide association mapping of septoria nodorum blotch resistance in Nordic winter and spring wheat collections. Theoretical and Applied Genetics 135:4169–4182
Lin M, Zhang DD, Liu SB, Zhang GR, Yu JM, Fritz AK, Bai GH (2016) Genome-wide association analysis on pre-harvest sprouting resistance and grain color in US winter wheat. Bmc Genomics 17
Liu F, Zhang XB, Lu CM, Zeng XH, Li YJ, Fu DH, Wu G (2015a) Non-specific lipid transfer proteins in plants: presenting new advances and an integrated functional analysis. J Exp Bot 66:5663–5681
Liu SB, Cai SB, Graybosch R, Chen CX, Bai GH (2008) Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. Theoretical and Applied Genetics 117:691–699
Liu SB, Sehgal SK, Lin M, Li JR, Trick HN, Gill BS, Bai GH (2015b) Independent mis-splicing mutations in TaPHS1 causing loss of preharvest sprouting (PHS) resistance during wheat domestication. New Phytol 208:928–935
Liu SB, Wang DF, Lin M, Sehgal SK, Dong L, Wu YY, Bai GH (2021) Artificial selection in breeding extensively enriched a functional allelic variation in TaPHS1 for pre-harvest sprouting resistance in wheat. Theoretical and Applied Genetics 134:339–350
Liu SP, Li L, Wang WL, Xia GM, Liu SW (2024) TaSRO1 interacts with TaVP1 to modulate seed dormancy and pre-harvest sprouting resistance in wheat. Journal of Integrative Plant Biology 66:36–53
Liu ST, Liu SW, Wang M, Wei TD, Meng C, Wang M, Xia GM (2014) A Wheat SIMILAR TO RCD-ONE Gene Enhances Seedling Growth and Abiotic Stress Resistance by Modulating Redox Homeostasis and Maintaining Genomic Integrity. Plant Cell 26:164–180
Liu YG, Ye NH, Liu R, Chen MX, Zhang JH (2010) H2O2 mediates the regulation of ABA catabolism and GA biosynthesis in seed dormancy and germination. J Exp Bot 61:2979–2990
Lohwasser U, Arif MAR, Börner A (2013) Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping. Biol Plantarum 57:663–674
Mares D, Rathjen J, Mrva K, Cheong J (2009) Genetic and environmental control of dormancy in white-grained wheat (Triticum aestivum L.). Euphytica 168:311–318
Mares DJ (1984) Temperature-Dependence of Germinability of Wheat (Triticum-Aestivum L) Grain in Relation to Pre-Harvest Sprouting. Australian Journal of Agricultural Research 35:115–128
Mares DJ, Mrva K (2014) Wheat grain preharvest sprouting and late maturity alpha-amylase. Planta 240:1167–1178
Martens H, Martens M (2000) Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). Food Qual Prefer 11:5–16

Martinez SA, Godoy J, Huang M, Zhang ZW, Carter AH, Campbell KAG, Steber CM (2018) Genome-Wide Association Mapping for Tolerance to Preharvest Sprouting and Low Falling Numbers in Wheat. Front Plant Sci 9

Matilla AJ (2024) Current Insights into Weak Seed Dormancy and Pre-Harvest Sprouting in Crop Species. Plants-Basel 13

Mcintosh RA, Hart GE, Gale MD (1989) Catalog of Gene Symbols for Wheat 1989 Supplement. Cereal Res Commun 17:77–89

Mohan A, Kulwal P, Singh R, Kumar V, Mir RR, Kumar J, Prasad M, Balyan HS, Gupta PK (2009) Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. Euphytica 168:319–329

Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theoretical and Applied Genetics 110:1315–1323

Moullet O, Bermúdez GD, Fossati D, Brabant C, Mascher F, Schori A (2022) Pyramiding wheat pre-harvest sprouting resistance genes in triticale breeding. Mol Breeding 42

Mroz T, Dieseth JA, Lillemo M (2023) Grain yield and adaptation of spring wheat to Norwegian growing conditions is driven by allele frequency changes at key adaptive loci discovered by genome-wide association mapping. Theoretical and Applied Genetics 136

Munkvold JD, Tanaka J, Benscher D, Sorrells M (2009) Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. Theoretical and Applied Genetics 119:1223–1235

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H (2011) A Wheat Homolog of MOTHER OF FT AND TFL1 Acts in the Regulation of Germination. Plant Cell 23:3215–3229

Nannuru VKR, Windju SS, Belova T, Dieseth JA, Alsheikh M, Dong YH, McCartney CA, Henriques MA, Buerstmayr H, Michel S, Meuwissen THE, Lillemo M (2022) Genetic architecture of fusarium head blight disease resistance and associated traits in Nordic spring wheat. Theoretical and Applied Genetics 135:2247–2263

Nguyen TN, Tuan PA, Sharma D, Ayele BT (2025) Alteration in the balance between ABA and GA signaling mediates genetic variation in induction and retention of dormancy during seed maturation in wheat. J Plant Physiol 304

Obendorf RL, Marcus A (1974) Rapid Increase in Adenosine 5'-Triphosphate during Early Wheat Embryo Germination. Plant Physiology 53:779–781

Obroucheva N, Antipova O (2000) The distinct controlling of dormancy release and germination commencement in seeds. In: Dormancy in plants: from whole plant behaviour to cellular control. Dormancy in plants: from whole plant behaviour to cellular control. Willingford, CABI Publishing, pp 35–46

Osa M, Kato K, Mori M, Shindo C, Torada A, Miura H (2003) Mapping QTLs for seed dormancy and the Vp1 homologue on chromosome 3A in wheat. Theoretical and Applied Genetics 106:1491–1496

Paterson AH, Sorrells ME, Obendorf RL (1989) Methods of Evaluation for Preharvest Sprouting Resistance in Wheat Breeding Programs. Can J Plant Sci 69:681–689

Perten H (1964) Application of Falling Number Method for Evaluating Alpha-Amylase Activity. Cereal Chem 41:127–+

Peska V, Garcia S (2020) Origin, Diversity, and Evolution of Telomere Sequences in Plants. Front Plant Sci 11

Rabieyan E, Bihamta MR, Moghaddam ME, Mohammadi V, Alipour H (2022) Genome-wide association mapping and genomic prediction of agronomical traits and breeding values in Iranian wheat under rain-fed and well-watered conditions. BMC Genomics 23

970 Rabinovich SV (1998) Importance of wheat-rye translocations for breeding modern cultivars of
971 Triticum aestivum L. (Reprinted from Wheat: Prospects for global improvement, 1998). Euphytica
972 100:323–340
973 Rajjou L, Gallardo K, Debeaujon I, Vandekerckhove J, Job C, Job D (2004) The effect of α-amanitin on
974 the Arabidopsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs
975 during germination. Plant Physiology 134:1598–1613
976 Rasul G, Humphreys GD, Wu JX, Brûlé-Babel A, Fofana B, Glover KD (2012) Evaluation of preharvest
977 sprouting traits in a collection of spring wheat germplasm using genotype and genotype environment
978 interaction model. Plant Breeding 131:244–251
979 Reddy LV, Metzger RJ, Ching TM (1985) Effect of Temperature on Seed Dormancy of Wheat. Crop Sci
980 25:455–458
981 Ruud AK, Begum MC, Uhlen AK, Yang E, Lin M, Mosleth EF, Dieseth JA, Lillemo M (2025) Quantitative
982 trait loci mapping of resistance to pre-harvest sprouting in the Norwegian spring wheat breeding line
983 T7347. Theor Appl Genet 138:159
984 Shao MQ, Bai GH, Rife TW, Poland J, Lin M, Liu SB, Chen H, Kumssa T, Fritz A, Trick H, Li Y, Zhang GR
985 (2018) QTL mapping of pre-harvest sprouting resistance in a white wheat cultivar Danby. Theoretical
986 and Applied Genetics 131:1683–1697
987 Shorinola O, Balcárková B, Hyles J, Tibbits JFG, Hayden MJ, Holusova K, Valárik M, Distelfeld A,
988 Torada A, Barrero JM, Uauy C (2017) Haplotype Analysis of the Pre-harvest Sprouting Resistance
989 Locus PhS-A1reveals a Causal Role of TaMKK3-A in Global Germplasm. Front Plant Sci 8
990 Shorinola O, Bird N, Simmonds J, Berry S, Henriksson T, Jack P, Werner P, Gerjets T, Scholefield D,
991 Balcárková B, Valárik M, Holdsworth MJ, Flintham J, Uauy C (2016) The wheat Phs-A1 pre-harvest
992 sprouting resistance locus delays the rate of seed dormancy loss and maps 0.3 cM distal to the PM19
993 genes in UK germplasm. J Exp Bot 67:4169–4178
994 Singh KL, Chaudhuri A, Kar RK (2015) Role of peroxidase activity and Ca2+ in axis growth during seed
995 germination. Planta 242:997–1007
996 Singh R, Matus-Cádiz M, Båga M, Hucl P, Chibbar RN (2010) Identification of genomic regions
997 associated with seed dormancy in white-grained wheat. Euphytica 174:391–408
998 Somyong S, Ishikawa G, Munkvold JD, Tanaka J, Benscher D, Cho YG, Sorrells ME (2014) Fine
999 mapping of a preharvest sprouting QTL interval on chromosome 2B in white wheat. Theoretical and
1000 Applied Genetics 127:1843–1855
1001 Strand E (1954) Studies on weather resistance of small grain species and varieties. Farm Crop
1002 Institute Agricultural College of Norway Report no 151
1003 Strand E (1989) Studies on seed dormancy in small grain species. II. Wheat. . Norw J Agric Sci 3:101–
1004 115
1005 Tai L, Wang HJ, Xu XJ, Sun WH, Ju L, Liu WT, Li WQ, Sun JQ, Chen KM (2021) Pre-harvest sprouting in
1006 cereals: genetic and biochemical mechanisms. J Exp Bot 72:2857–2876
1007 Tang H, Wang HB, He MJ, Zhang MY, Hu YS, Li Z, Ren TH, Ren ZL, Yan BJ (2017) QTL analysis for wheat
1008 falling number in a recombinant inbred line population segregated with 1BL/1RS translocation in a
1009 rainfed agricultural area of China. Euphytica 213
1010 Torada A, Ikeguchi S, Koike M (2005) Mapping and validation of PCR-based markers associated with
1011 a major QTL for seed dormancy in wheat. Euphytica 143:251–255
1012 Torada A, Koike M, Ogawa T, Takenouchi Y, Tadamura K, Wu JZ, Matsumoto T, Kawaura K, Ogihara Y
1013 (2016) A Causal Gene for Seed Dormancy on Wheat Chromosome 4A Encodes a MAP Kinase Kinase.
1014 Curr Biol 26:782–787
1015 Turner SD (2018) qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots.
1016 Journal of Open Source Software 3:731

Tyagi S, Gupta PK (2012) Meta-analysis of QTLs involved in pre-harvest sprouting tolerance and dormancy in bread wheat. Triticeae Genomics and Genetics 3:9–24
Walker-Simmons M (1987) ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiology 84:61–66
Wang H, Wang W, Guo Y, Zhang M, Simmonds J, Zheng Q, Hou J, Liu X, Bie X, Zhang X, Zhang X, Uauy C, Lu A, Chu C, Tian Z, Xiao J (2024) Natural variations of TaMYB7-A1 regulate PHS resistance and specify wheat geographic adaptation-.
Wang JB, Zhang ZW (2021) GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. Genom Proteom Bioinf 19:629–640
Wang XY, Liu H, Liu GN, Mia MS, Siddique KHM, Yan GJ (2019) Phenotypic and genotypic characterization of near-isogenic lines targeting a major 4BL QTL responsible for pre-harvest sprouting in wheat. Bmc Plant Biol 19
Watahiki M, Trewavas A (2019) Systems, variation, individuality and plant hormones. Prog Biophys Mol Bio 146:3–22
White J, Sharma R, Balding D, Cockram J, Mackay IJ (2022) Genome-wide association mapping of Hagberg falling number, protein content, test weight, and grain yield in UK wheat. Crop Sci 62:965–981
Wold S, Martens H, Wold H (1983) The Multivariate Calibration-Problem in Chemistry Solved by the Pls Method. Lect Notes Math 973:286–293
Worrall D, Liang YK, Alvarez S, Holroyd GH, Spiegel S, Panagopulos M, Gray JE, Hetherington AM (2008) Involvement of sphingosine kinase in plant cell signalling. Plant J 56:64–72
Wu FH, Chi Y, Jiang ZH, Xu YY, Xie L, Huang FF, Wan D, Ni J, Yuan F, Wu XM, Zhang YY, Wang L, Ye R, Byeon B, Wang WH, Zhang S, Sima M, Chen SP, Zhu MH, Pei JS, Johnson DM, Zhu S, Cao XQ, Pei C, Zai ZJ, Liu YH, Liu TY, Swift GB, Zhang WG, Yu M, Hu ZL, Siedow JN, Chen X, Pei ZM (2020) Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in Arabidopsis. Nature 578:577–581
Xue TT, Wang D, Zhang SZ, Ehlting J, Ni F, Jakab S, Zheng CC, Zhong Y (2008) Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. Bmc Genomics 9
Yang R, Harrison MT, Yang YM, Wang CH, Shabala S, Huang MX, Zhao CC, Zhou MX, Sun CM, Liu K (2025) Pre-harvest sprouting in cereals: Global incidence, impacts and mitigation strategies. Field Crop Res 333
Yazdani M, Zallot R, Tunc-Ozdemir M, de Crécy-Lagard V, Shintani DK, Hanson AD (2013) Identification of the thiamin salvage enzyme thiazole kinase in Arabidopsis and maize. Phytochemistry 94:68–73
Zhang BW, Yang BS, Wan XN, Ma X, Lyu KD, Wang H, Yang SY, Zhang HH, Hao SN, Ma J, Sun GZ (2025) Evaluation of TaMFT-3A and TaMKK3-4A alleles on wheat pre-harvest sprouting. Front Plant Sci 16
Zhao CH, Cui F, Wang XQ, Shan SC, Li XF, Bao YG, Wang HG (2012) Effects of 1BL/1RS translocation in wheat on agronomic performance and quality characteristics. Field Crop Res 127:79–84
Zhou Y, Tang H, Cheng MP, Dankwa KO, Chen ZX, Li ZY, Gao S, Liu YX, Jiang QT, Lan XJ, Pu ZE, Wei YM, Zheng YL, Hickey LT, Wang JR (2017) Genome-Wide Association Study for Pre-harvest Sprouting Resistance in a Large Germplasm Collection of Chinese Wheat Landraces. Front Plant Sci 8
Zhu YL, Wang SX, Wei WX, Xie HY, Liu K, Zhang C, Wu ZY, Jiang H, Cao JJ, Zhao LX, Lu J, Zhang HP, Chang C, Xia XC, Xiao SH, Ma CX (2019) Genome-wide association study of pre-harvest sprouting tolerance using a 90K SNP array in common wheat (Triticum aestivum L.). Theoretical and Applied Genetics 132:2947–2963
Zhu YL, Wang SX, Zhang HP, Zhao LX, Wu ZY, Jiang H, Cao JJ, Liu K, Qin M, Lu J, Sun GL, Xia XC, Chang C, Ma CX (2016) Identification of major loci for seed dormancy at different post-ripening stages after harvest and validation of a novel locus on chromosome 2AL in common wheat. Mol Breeding 36

Zuo JH, Lin CT, Cao H, Chen FY, Liu YX, Liu JD (2019) Genome-wide association study and quantitative trait loci mapping of seed dormancy in common wheat (Triticum aestivum L.). Planta 250:187–198

Table text

Table 3. Results from supervised multivariate analyses of all genotypes for the markers presented in Table 2 with FN or GI as the responses. The frequencies of each alternative marker allele in MASBASIS and the validation data (A Norwegian breeding population). The PLS loadings of component 1 and component 2 are the most relevant linear projections of the data. Results of validation by Jackknife of each allele are marked by bold of the loadings.

Table text to Supplementary Tables

Table S1. MASBASIS A. Phenotype presented as LSmeans for A. FN and B. GI from individual trials.

Table S2. Validation data**.** Phenotype data by falling number (FN) and Germination index (GI

Table S3. MASBASIS. Complete results from GWAS of 25K SNP chip and 35K SNP chip. Published markers with the maximal distance equal to 10 Mbp between the first and the last marker of a QTL as obtained from "Wheat@URGI" (https://wheat-urgi.versailles.inra.fr/) online portal are presented in the table. Each sheet gives the trait group (high FN, medium FN, low FN or GI) where it is tested, the marker names, chromosome position, the frequency at which the second most common allele occurs in a given population (minor allele frequency, maf), the significance validation, and the effect

a) high FN group (FN>300 s), 25K
b) medium FN group (FN:200-300 s), 25K
c) low FN group (FN<200 s), 25K
d) GI, 25K
e) individual_trials, FN, 25K
f) individual_trial, GI, 25K
g) high FN group (FN>300 s), 35K
h) medium FN group (FN:200-300 s), 35K
i) low FN group (FN<200 s), 35K
j) GI, 35K
k) individual_trials, FN, 35K
l) individual_trial, GI, 35K

Table S4. Information that supplements Table 2 on the significant QTL.

Table S5. Composition of SNP markers in the validation data.

Table S6. Results from supervised multivariate analyses of all markers presented in Table 2 with FN or GI as the responses applied among the non-adapted genotypes and among the adapted genotypes. The frequencies of each

alternative marker allele are provided in MASBASIS and the validation data (A Norwegian breeding population). The PLS loadings of component 1 and component 2 are the most relevant linear projections of the data. Results of validation by Jackknife of each allele are marked by bold of the loadings.

Table S7. Table S7. Markers included in allele stacking of MASBASIS and Validation data.

Table S8. Genes identified by KnetMiner located in the range of +/- 2 Mbp of the peak markers in each significant QTL.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.MASBASISphenotypedata.xlsx
- TableS2.Validationdata.PhenotypeFNandGI..xlsx
- TableS3.GWASresultsMASBASIS.xlsx
- TableS4.Extrainformationonthemostsignificantmarkers.xlsx
- TableS5.ValidationdataSignificantMarkersfromTable2availableinthevalidationdata.xlsx
- TableS6.PLSFNGINadandAdinMASBASIS.xlsx
- TableS7.MarkersincludedinallelestackingofMASBASISandValidationdata.xlsx
- TableS8.KnetMiner.xlsx