Regional jordbruksforskning för norra Sverige (RJN) –

Progress report 2023

Project: RJN 1/2022. NorMal - Utveckling av nya maltkornsorter för norra Sverige

Project leader: Fluturë Novakazi

The development of a barley cultivar suitable for malting and adapted to the Nordic conditions could increase the economic return for farmers, create the possibility of a new industry for the northern regions, as well as enhance rural development.
However, the analysis of malting traits is time consuming, laborious and requires a relatively high amount of seed. Therefore, it can only be done on a small number of lines within the breeding programme. Additionally, one has to wait until harvest to perform those analyses, which postpones selection of lines for malting traits to the end of the growth cycle. The use of genomic selection in the breeding programme will allow for screening of more lines for malting traits and selection in an earlier plant stage. Thereby decrease costs for lab analyses and the time of each selection cycle.

Project progress

The project proceeded according to the proposed plan with a minor delay of the development of the prediction model, since the micro-malting was conducted later as planned (July 2023) (Table 1).

Table 1 Gantt schedule for the project "NorMal - Utveckling av nya maltkornsorter för norra Sverige".

WP	Uppgift	2022 sommar	2022 höst	2022 vinter	2023 vår	2023 sommar	2023 höst	2023 vinter
WP 1 - Fältförsök och utvärdering av agronomiskt egenskaper och mältnings-egenskaper	1.1 Fältförsök och utvärdering av agronomiska egenskaper							
	1.2 Analys av mältningsegenskaper							
WP 2 - Genotypning och genomiska prediktionsmodeller	2.1 Genotypning							
	2.2 Genomiska prediktionsmodeller							rapport

Work package 1 – Field trials and assessment of agronomic and malting traits (Leader: Firuz Odilbekov)

A set consisting of 273 spring barley breeding lines plus one check ("Anneli") was grown in yield plots in 2022 under field conditions in Lännäs. The trial was set up in an augmented design with nine blocks and nine checks and 39 plots per block.

After harvest in September 2022, 2kg of seed per breeding line were sent to the laboratory in Svalöv and analysed for malting quality associated traits (basic traits). All lines were analysed for the following traits: seed size >2.8mm, 2.5 – 2.8mm, 2.2 – 2.5mm and <2.2mm, undamaged kernels (whole grains), thousand kernel weight, germination rate at 4mL and 8mL, water content, hectolitre weight (weight by volume) and raw protein content (Figure 1).

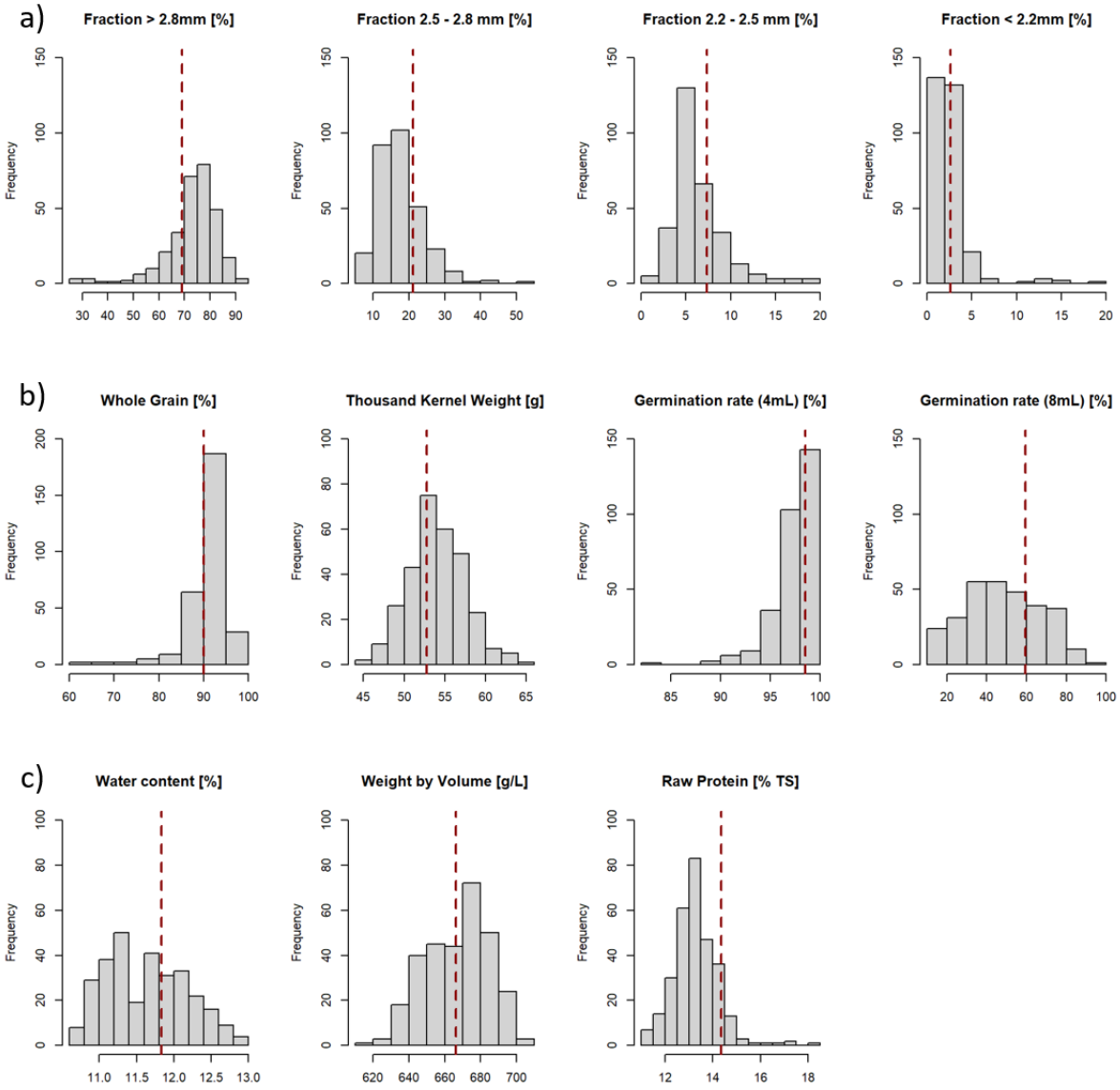

Figure 1 Malting quality associated traits assessed in a spring barley set consisting of 274 breeding lines and grown under field conditions in 2022 in Lännäs, Sweden. Dark red, dashed lines represent the malting cultivar 'Anneli', which was used as a check.

Based on the analyses of the basic traits (malting quality associated traits), 50 lines were selected to undergo micro-malting. For the selection, the 273 lines were grouped according to their values for whole grains, thousand kernel weight, hectolitre weight, water content and raw protein.
The mean, maximum and minimum values of the above-mentioned traits for the 50 selected lines can be obtained from Table 2.

Table 2 Mean, maximum and minimum values for whole grain (fullkorn), hectolitre weight (rymvikt), raw protein (raprotein), water content (vattenhalt), and thousand kernel weight (tusenkornsvikt) for 50 selected barley lines.

	Fullkorn	Rymdvikt	Raprotein	Vattenhalt	Tusenkornsvikt
mean	90.75	665.82	13.16	11.78	54.66
max	98.20	710.00	18.20	12.90	64.28
min	68.80	638.00	11.20	10.80	45.65

Malt quality analysis of the selected 50 lines was done in-house at Lantmännen's laboratory in July of 2023. For this, 50g of grains of each line were malted under standardized conditions. Subsequently, the following parameters were analysed: moisture, friability, filtration time, wort density, viscosity, malt extract, wort clarity, wort colour, wort odour, and wort density. The results of the micro-malting analysis can be seen in Figure 2 and Table 3.

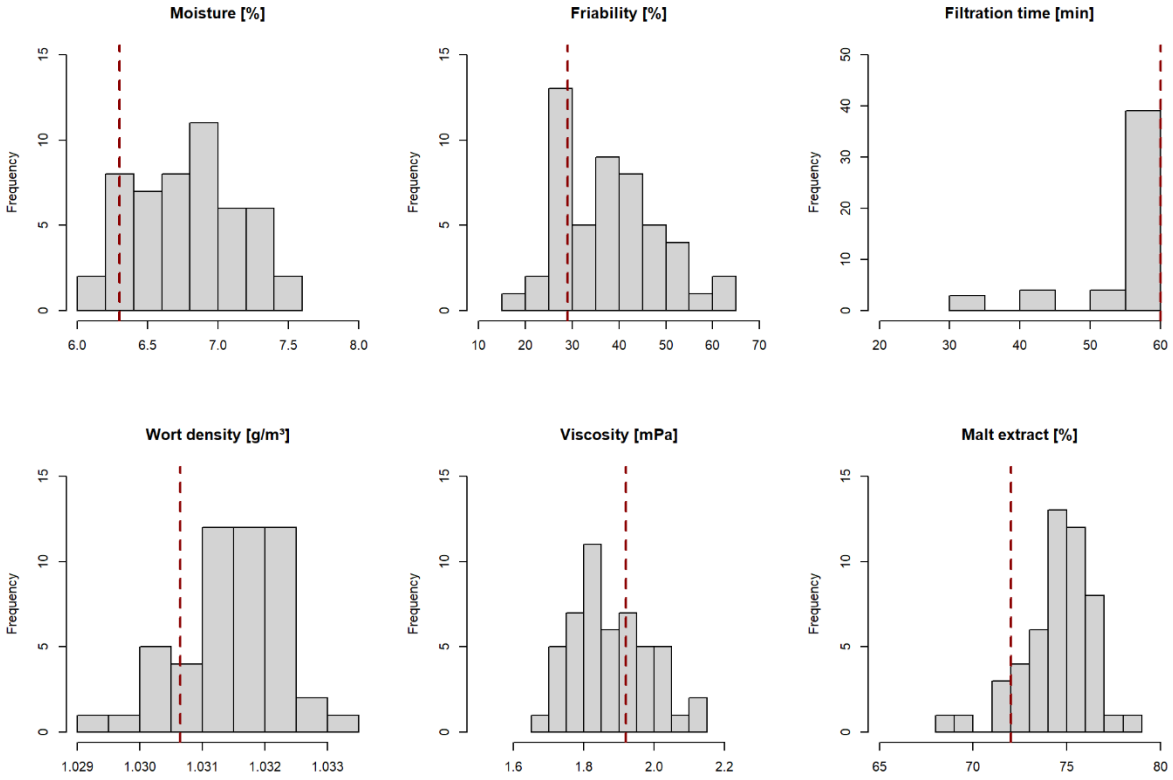

Figure 2 Micro-malting analysis assessed in a spring barley set consisting of 50 breeding lines and grown under field conditions in 2022 in Lännäs, Sweden. Dark red, dashed lines represent the malting cultivar 'Anneli', which was used as a check.

The moisture content is important for shelf life and milling. Malts with low moisture content are brittle and prone to breakage, while high moisture malts become slack. Optimum moisture contents for base malts range between 3 to 6%, however, for Pilsen Malt the optimum contents are slightly higher. The analysed lines showed moisture contents between 6.1 and 7.5%, hence, they would be suitable for Pilseners (Figure 2, Table 3).

Friability is defined as how easily something crushes under pressure. The friability in the malting analysis is a measure of malt modification and how hard the grains are. The more modified the malt is, the easier it will crush. For this analysis 5g of malt is placed into a sieve drum, where a pressure arm presses the malt against the sieve. The portion of malt that falls through is friable. The check cultivar "Anneli" showed a friability of 29%, whereas most lines showed friability values of over 35% (Figure 2). However, desirable friability values are in order of 80% and higher.

The malt is ground to a very fine powder with a standardised coarse grind. The powder is mashed, which simulates the brewing process and subsequently, filtration time, wort density and viscosity can be measured.

In the filtration step, the mashed wort (water-soluble extract) is separated from the grains and is necessary to obtain a high-quality extract yield. Shorter times are desirable. In the analysed samples, most genotypes showed filtration times exceeding 60 minutes, which was the maximum time of observation (Figure 2).

The density of a liquid is measured with a hydrometer, which floats in the liquid. Water has a density of 1.000. The wort density is mostly influenced by the sugar content of the wort, i.e. the more dissolved sugars, the higher the density. Sugar is necessary in any fermentation process to produce alcohol and therefore essential in the brewing process. With the wort density, the brewer can estimate when fermentation is complete and the alcohol content of the beer. Higher densities can be achieved by adding more grains to the recipe. The check cultivar "Anneli" had a wort density of 1.03064, but most lines showed a density between 1.031 and 1.033 (Figure 2), suggesting to be more efficient for brewing than "Anneli", as less grain would be necessary to achieve the same alcohol content.
High wort viscosities can slow down the lautering and influence beer quality and taste negatively. It is influenced by the β-glucan content. β-glucans are cell wall components and have to be degraded so starch becomes accessible for enzymes like α-amylase, β-amylase, limit-dextrinase and α-glucosidase. If starch cannot be accessed by enzymes and degraded to yeast available sugars, malt extract and alcohol yield are negatively impacted. Hence, higher wort viscosities are due to insufficient cell wall decomposition and negatively influence filtration of the wort and beer. Lower values are due to too low break down of the cell wall material and cause poor foam stability and beer taste. Additionally, high β-glucan content in the wort leads to increased haze formation in the end product. Wort viscosity of the analysed lines was between 1.7 and 2.13 mPa, with a mean at 1.88 mPa, which is in the range of typical wort viscosities (Table 3).
The wort density and viscosity are used to calculate the malt extract, which should be as high as possible. The malt extract of the analysed barley lines ranged from 68.7 to 78.5%, with "Anneli" showing a malt extract of 72% (Figure 2, Table 3).

Table 3 Number of entries (n), mean, standard deviation (sd), minimum (min), maximum (max) and standard error (se) values for parameters analysed in micro-malting of 50 spring barley breeding lines.

	n	mean	sd	min	max	se
Filtration time [min]	50	56.60	7.98	30.00	60.00	1.1288
Wort density [g/m³]	50	1.03	0.00	1.03	1.03	0.0001
Moisture [%]	50	6.84	0.37	6.10	7.50	0.0520
Viscosity [mPa]	50	1.88	0.11	1.69	2.13	0.0155
Friability [%]	50	38.00	10.44	19.90	64.50	1.4761
Whole grains [%]	50	23.95	12.06	2.50	47.00	1.7056
Malt extract [%]	50	74.57	1.90	68.70	78.50	0.2691

The wort clarity is visually scored. About half of the lines (n = 26), showed a clear wort, 17 had a hazy or cloudy wort and seven had an opaque wort (data not shown). A clear wort is desirable, as it gives a clearer product and better quality. The wort colour is scored on a visual scale based on the Standard Reference Method (SRM) for scoring beer colour. Based on the colour of the wort, one can infer for which kind of beer the malt can be used. Malts with lighter colours are suitable for lighter beers, like lagers and pilseners, darker malt can be used for dark lagers, porters or stouts. In our samples 17 lines had a wort colour of 2, 23 had a wort colour of 3 and the remaining lines lay between 2 and 3 (data not shown). This means the malts are suitable for brewing pale lagers or German pilseners. There was no malt that exhibited an unwanted odour (data not shown).

Work package 2 – Genotyping and genomic prediction model (Leader: Alf Ceplitis)

Seeds of all 273 barley lines were sent to SGS Institute Fresenius GmbH, TraitGenetics Section, Gatersleben, Germany, where DNA was extracted for genotyping with the 50k barley iSelect SNP chip at the beginning of 2023. Among the 44,400 SNP markers, 34,841 markers were polymorphic in the panel. After filtering for >20% missing values and >5% MAF, 29,260 polymorphic SNP markers

remained for further analyses. Based on the kinship, the breeding lines did not show any strong population structure, except for seven breeding lines, which seemed to be more closely related to each other than to the rest (Figure 3).

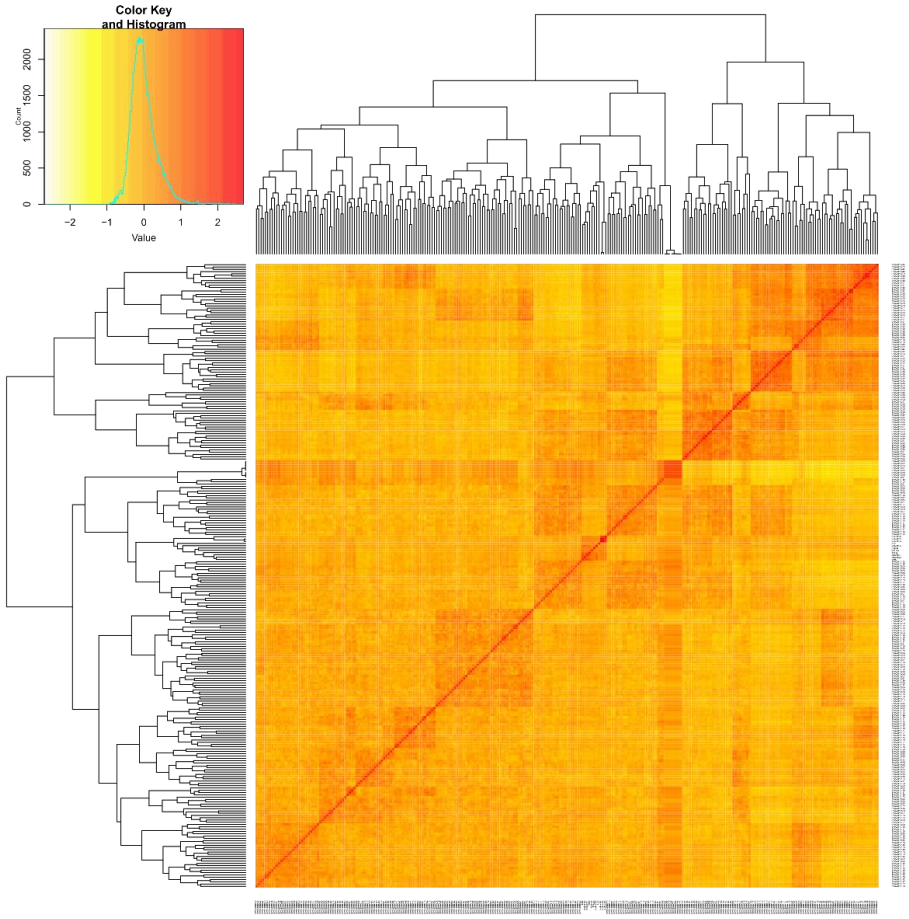

Figure 3 Kindship heatmap based on vanRaden for 273 spring barley breeding lines genotyped with the 50k iSelect SNP chip.

Since full malting analysis was performed on only 50 lines, which is normally too small a number for the development of reliable prediction models, we examined the correlations between the basic grain/malting traits (see above) and the full malting traits. If one or more basic traits show a strong correlation to more complex malting traits, it might be more feasible to develop genomic prediction model for such traits as a proxy for malting quality. We found strong correlations between grain size >2.8 mm, hectolitre weight and raw protein content on the one hand, and wort density and malt extract on the other. There was also a significant, but slightly weaker, correlation between protein content and friability (Table 4).

Table 4 Correlations between basic traits and quality traits from full malting analysis. Statistically significant correlations are bold.

Basic trait	Wort density	Malt extract	Friability
Grain size >2.8 mm	**0.70**	**0.74**	0.18
Hectolitre weight	**0.71**	**0.69**	0.12
Raw protein content	**-0.81**	**-0.81**	**-0.55**

Given the observed correlations, we developed and validated genomic prediction models for grain size >2.8 mm, hectolitre weight and raw protein content. Prediction models were constructed by ridge-regression BLUP where SNP alleles were numerically coded as 1, 0, and -1 for homozygotes for the maternal allele, heterozygotes, and homozygotes for the paternal allele, respectively. Missing alleles were imputed by the mean for each marker.

Genomic prediction accuracy (PA) was determined by a cross-validation procedure where 88% of the panel was used as training set and the remining 12% as validation set. The training set was chosen by randomly sampling 250 lines from the total of 274 lines. PA was defined as the Pearson correlation coefficient between the predicted and observed trait values in the validation. The sampling procedure was repeated 500 times and an average PA was calculated. The average PA for the three traits was high: 0.65 for grain size, 0.67 for protein content, and 0.74 for hectolitre weight (Figure 4).

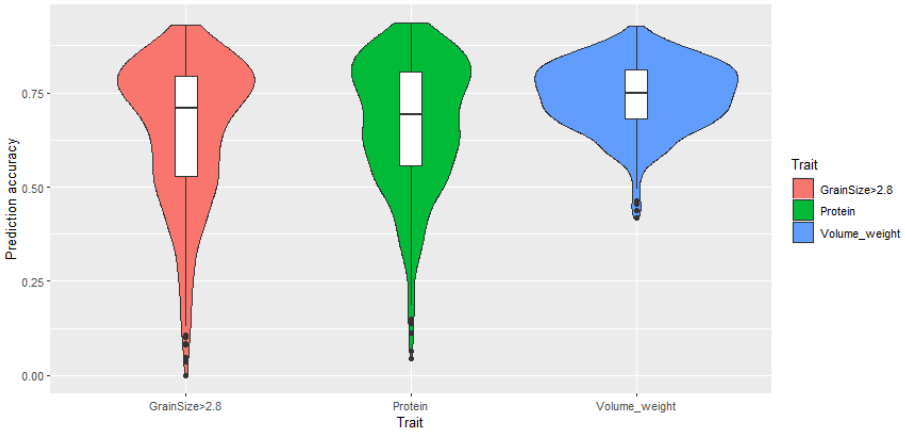

Figure 4 Distribution of genomic prediction accuracy for each of three quality traits.

Conclusion

Grain size (> 2.8mm) and hectolitre weight showed a significant positive correlation with the important malting traits wort density and malt extract. Protein content showed a significant negative correlation with wort density, malt extract and friability, which is expected, since a too high protein content is unfavourable in the malting process. However, based on the developed prediction models, these three basic traits, i.e. grain size, protein content and hectolitre weight showed high prediction accuracies and are therefore good indicators for malting quality. Thus, basic traits, which are easier and cheaper to assess can be used for selection of breeding lines intended to become malting cultivars.