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Leaf protein extractability in sugar beet: Genetic control and breeding potential for dual-purpose cultivars

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ABSTRACT

Context and objective: Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is primarily cultivated for sugar, but its abundant leafy biomass also represents a promising source of plant protein. Achieving high protein extraction yields without compromising sugar yield is a key to economic viability. This study evaluated the genetic potential of sugar beet for leaf protein production and identified genomic regions associated with traits relevant to leaf protein extractability.

Methods: Across four field trials, 200 sugar beet hybrids were evaluated for traits related to leaf protein extraction (leaf protein content, extractable protein content, extractable RuBisCO, protein extractability, and leaf yield), alongside root yield and sugar content. Correlations between leaf and sugar traits were assessed, and a genome-wide association study (GWAS) was conducted to identify associated genetic markers.

Results and conclusions: Sugar yield showed only a week correlation with leaf protein yield, suggesting that leaf protein can be enhanced without a significant trade-off in sugar yield. The GWAS identified 48 quantitative trait loci (QTL) regions associated with leaf protein content and related traits, with several markers explaining over 10 % of the phenotypic variance, suggesting promising selection targets. Candidate genes were linked to photosynthesis, amino acid transport, and cell wall metabolism, providing insights into the genetic control of leaf protein content and extractability in sugar beet.

Significance: These findings highlight the potential to breed sugar beet cultivars that combine high sugar yield with improved leaf protein extractability. The identified genetic markers provide useful tools to accelerate the development of dual-purpose cultivars, supporting the sustainable valorization of sugar beet biomass.

1. Introduction

The global demand for plant-based protein is rapidly increasing, driven by the need to transition from animal-derived sources to more sustainable alternatives with a lower environmental footprint. Among potential sources, green leaves represent an abundant yet underutilized reservoir of plant protein, available in agricultural by-products. Leaf protein isolates not only provide essential amino acids but also exhibit superior functional properties, making them particularly attractive for food applications (Ducrocq et al., 2022; Martin et al., 2019, 2014). The valorization of green leaf biomass thus holds promise to enhance the efficiency and sustainability of food systems while offering farmers additional economic returns.

Approximately 80 % of leaf proteins are localized in the chloroplast, with soluble proteins in the stroma and insoluble proteins in the thylakoid membranes (Fiorentini and Galoppini, 1983). Among these, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the most abundant, representing nearly half of the soluble protein fraction. Owing to its balanced essential amino acid composition and favourable functional properties, RuBisCO isolates are increasingly recognized as promising plant-based food ingredients (Barbeau and Kinsella, 1988; Martin et al., 2019).

Sugar beet (*Beta vulgaris* L.) is primarily cultivated for sugar production from storage roots, yet it produces a substantial leaf biomass that remains underutilized. On average, total dry matter yield reaches 28.5 t ha^{-1}, of which ~25 % corresponds to leaves (Starke and

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Hoffmann, 2014). These leaves can contain 16–22.8 % crude protein (Lammens et al., 2012), making them a potentially valuable source of plant protein. However, current extraction yields remain low; for example, only ~6 % of total protein was recovered as RuBisCO in sugar beet leaves (Tamayo Tenorio et al., 2016).

Protein extraction efficiency is influenced by multiple biological and technical factors, including cell wall composition, phenolic compounds, and protease activity, as well as the extraction protocol applied (Kiskini et al., 2016; Akyuz and Ersus, 2021; Kleuter et al., 2024). Environmental and agronomic factors also play a role. Younger leaves generally yield proteins of higher quality compared to older leaves (Kiskini et al., 2016). Nitrogen fertilization, timing of application, and harvest stage influence both leaf protein quality and recovery. Yet, in sugar beet, nitrogen management must balance potential benefits for leaf protein without any trade-off in root yield and sugar quality, since excessive nitrogen reduces sucrose concentration (Dutton & Huijbregts, (2006); Hergert, 2011; Wang et al., 2025).

Genotypic variation further affects leaf protein content and extractability. For instance, alfalfa accessions differ significantly in crude and soluble protein levels (Lin et al., 2025), while quinoa varieties show crude protein contents ranging from 11 to 20 % and soluble protein between 2.4–16.1 % of dry matter (Pérez-Vila et al., 2025). Despite such evidence in other crops, little is known about the genetic diversity of leaf protein traits in sugar beet. Historically, breeding has targeted sugar yield and disease resistance (Hoffmann and Kenter, 2018), often at the expense of leaf biomass and cell wall composition. Whether modern high-sugar varieties are suitable for protein extraction, or whether breeding for improved protein yield may conflict with sugar yield selection, remains largely unexplored.

Understanding the phenotypic diversity of leaf protein extraction yield in sugar beet, and its relationship with root and sugar traits, is therefore critical to assess potential trade-offs and identify cultivars with dual-purpose value. Moreover, advances in genomic tools offer new opportunities to accelerate breeding. Marker-assisted selection (MAS) can be guided by genome-wide association studies (GWAS), which have successfully identified marker–trait associations for sugar content and root yield in sugar beet (Wang et al., 2024; Wurschum et al., 2011). Although no GWAS for leaf protein traits has yet been reported in sugar beet, studies in alfalfa and soybean have detected loci linked to protein content and extractability (Biazzi et al., 2017; Montes et al., 2022). This suggests that similar genetic markers may exist in sugar beet.

To date, there has been no systematic evaluation of genetic diversity in leaf protein content and extractability across a large sugar beet panel. The present study therefore aims to address this gap by (i) quantifying total and extractable leaf protein content, (ii) estimating protein extractability, (iii) assessing heritability of protein traits, (iv) analysing correlations between leaf protein, root, and sugar traits to test for trade-offs, and (v) conducting a genome-wide association analysis to uncover the genetic basis of variation in leaf protein traits.

2. Methods

2.1. Plant material

The plant material used in this study comprised 200 *Beta vulgaris* hybrids from sugar beet breeding company KWS SAAT SE & Co. KGaA (Einbeck, Germany). The hybrids were evaluated in routine sugar beet breeding trials conducted across four locations in 2022 and 2023 (Table 1). To account for potential field heterogeneity, a randomized complete block design (RCBD) with two blocks was implemented. Each plot consisted of three rows, with 30 plants per row, resulting in a total of 90 plants per plot.

Table 1
Field trials. Overview of field trials, including trial code, location and date of sowing, leaf, and beet harvest.

Trial	Location	Sowing	Leaf harvest	Beet harvest
NL22	Zwolle (NL)	19/4/2022	12/9/2022	21/9/2022
FR22	Lens (FR)	13/4/2022	19/9/2022	30/9/2022
NL23	Zwolle (NL)	2/5/2023	11/9/2023	27/9/2023
FR23	Lens (FR)	15/04/2023	4/9/2023	23/10/2023

2.2. Sample collection

Leaf samples were collected at the end of the growing season (Table 1). Leaves were harvested without the petiole and placed in 50 × 80 cm monofilament net bags. In 2022, all leaves from 10 plants in the middle row of each plot were collected. In 2023, 3–4 young fully expanded leaves were sampled from each plant in the middle row.

2.2.1. Sample processing

The bulk leaf sample was mixed and split into two subsamples (A and B). Subsample A was dried and used to determine the dry matter and total protein content (LPC) of the leaves. Subsample B was processed fresh and used to determine the amount of extractable protein (ELPC). To determine extractable protein and protein extractability of the total sample, we used the parameters of both subsamples (A and B). In 2022, the dry leaf yield per plant was determined by measuring the fresh weight of the bulk leaf sample before splitting. Leaf yield (LY) was calculated using the fresh weight of the bulk leaf sample, the dry matter content of subsample A, and the number of plants sampled (Eq. 1).

$$Leaf\ yield = \frac{Fresh\ weight(bulk) * Dry\ matter\ proportion(A)}{Nplants} \quad (1)$$

After splitting of the bulk leaf sample, we placed subsample A back into the monofilament net bag and recorded the fresh weight of the leaves. The leaves were then dried at 50 °C for 48 h. The dried leaves were weighed, milled (1 mm) and stored in airtight containers. The fresh and dry weight were used to calculate the dry matter content of the leaves. Near-infrared spectral absorbance (400–2500 nm) of the dried and milled leaf samples were recorded with a FOSS NIRs analyzer (FOSS DS2500). NIRs based prediction models were previously developed in (Rijken et al., 2025), and used in this study to predict total leaf protein content (LPC), and extractable RuBisCO content (ER) of the samples.

Subsample B was collected in airtight and lightproof aluminum zip-bags (30 × 50 cm, 9 l) and stored at 4 °C to minimize degradation of the leaves. Within three days after harvest, a twin screw press (Angel Juicer 7500, Angel Juicer Co. LTD, South-Korea) was used to press the leaves. The juice was collected and weighed to determine the percentage of juice pressed from the leaves. A 2 ml subsample of the juice was taken and centrifuged at 20,000 G for 45 min to precipitate any solids. A subsample of the supernatant (500 µl) was transferred into a tin capsule (20 × 8 mm, Elemental microanalysis), and dried for 48 h at 60 °C. The nitrogen content of the supernatant was measured through combustion of the dried tin capsules in a rapid N-exceed nitrogen and protein analyzer (Elementar).

The extractable protein content and protein extractability of the bulk leaf sample was calculated by using parameters measured on both subsamples separately. For this calculation, the dry matter and total protein content of subsample A were combined with the percentage of juice and the supernatant protein of subsample B. Extractable protein (g/100 g dried leaves) was determined as the ratio between protein in the juice and the dry matter content of the leaves, as shown in Eq. (2).

$$Extractable\ protein = \frac{(Percentage\ of\ juice(B) * Protein\ in\ supernatant(B))}{Dry\ matter\ content(A)} \quad (2)$$

The extracted protein was then expressed as a percentage of total protein to obtain protein extractability (g/100 g protein) in Eq. (3):

$$Protein\ extractability = \frac{Extracted\ protein}{Total\ protein} * 100 \quad (3)$$

It should be noted that the extractable leaf protein content (ELPC), as measured by nitrogen combustion may also contain non-protein nitrogen (NPN) compounds, whereas the extractable RuBisCO (ER) assay provides more protein-specific measure.

Besides the leaves, the sugar beet roots were also collected and measured for sugar content and root yield at KWS SAAT SE&Co KGaA. For this, the beets were lifted and cleaned before weighing. Then the beets are crumbled and Near-InfraRed absorption spectra were measured using a Polytec PSS/PAS 1720 (850–1650 nm) Spectrometer system. Phenotypes were predicted using in-house calibration models.

2.3. Statistical analysis of the phenotype data

Phenotypes were adjusted for spatial variation using the SpATS model, which applies two-dimensional smoothing over rows and columns (Rodríguez-Álvarez et al., 2018). Genotypes were treated as a fixed effects to obtain the best linear unbiased estimates (BLUEs) per trial. A two-stage analysis then combined these BLUEs across trials (Eq. 4) using the lme4 package in R (Bates et al., 2015).

$$Y = Intercept + Genotype + Environment + Residual \quad (4)$$

To assess the genetic determination of the studied traits, plot-based heritability was calculated both within and across trials. For this, variance components for Genotype, Genotype × Environment interaction (G×E), and Residual were estimated by fitting models 5 and 6 on the phenotype data.

$$Y = Intercept + Genotype + Block + Residual \quad (5)$$

$$Y = Intercept + Genotype + Environment + Block + GEI + Residual \quad (6)$$

where Y is the response variable, Intercept represents the overall mean, Genotype is a random effect of the accession, Environment is a random effect of the trial, Block is a random effect nested within the trial, GEI represents the random effect of the genotype-by-environment interaction, and Residual is the error term. Broad-sense heritability (H^2) was then calculated using the following formula:

$$H^2 = VG \Big/ \left(VG + \frac{VGEI}{nE} + \frac{V\varepsilon}{nE * nBlock}\right) \quad (7)$$

where VG is the variance component of genotype, VGEI is the variance component of the genotype-by-environment interaction, Vε is the variance component of the residual, nE represents the number of environments, and nBlock the number of blocks in the trial design.

2.4. Sugar and protein yield parameters

To compare the yield of the main harvest component (sugar) to the yield of leaf protein, the adjusted genotypic means across trials were used to calculate the total available leaf protein (TLP*), protein yield (PY*), RuBisCO yield (RubY*), and sugar yield (SY*) expressed as dt/ha. Sugar yield was calculated using Eq. (8).

$$Sugar\ yield = Root\ yield * Sugar\ content = \quad (8)$$

TLP*, PY* and RubY* (dt/ha) were calculated for each accession using Eqs. 9, 10 and 11.

$$Total\ leaf\ protein = LY * Nplants * \frac{LPC}{100} \quad (9)$$

$$Protein\ yield = LY * Nplants * \frac{ELPC}{100} \quad (10)$$

$$RuBisCO\ yield = LY * Nplants * \frac{ER}{100} \quad (11)$$

Where leaf yield (LY) is the adjusted genotypic mean obtained across-trials for leaf dry matter per plant. Nplants was set at 90,000, an optimal amount of plants/ha (KWS, (2025)), LPC, ELPC and ER represent the adjusted genotypic mean of leaf protein content, extractable leaf protein content and extractable RuBisCO content.

The relationships between sugar and leaf protein yield parameters were investigated through correlation analysis, and principal components analysis (PCA). Correlation analysis was performed in R, using the chart.Correlation function from PerformanceAnalytics R package (Peterson and Carl, 2024). PCA was conducted using the prcomp function in R, and visualized using the fviz_pca_biplot function from the factoextra package in R (Kassambara and Mundt, 2020).

2.5. Genotyping and imputation

The genotypic data used in this study was provided by KWS SAAT SE & Co KGaA. Plant DNA was extracted using silica-membrane technology following the NucleoSpin® 96 Plant II protocol from Macherey-Nagel. Samples were genotyped with a customized in-house SNP chip, generating a raw dataset of 10,780 SNP markers spanning the entire sugar beet genome. The dataset included mapping information, with SNPs annotated by chromosome and genetic position in centimorgans.

Genotype imputation was performed using the codeMarkers function in statgenGWAS package in R (van Rossum and Kruijer, 2022). Quality control steps were applied to filter out markers with more than 10 % missing values, duplicate markers, and markers with a minor allele frequency below 5 %. Additionally, accessions with more than 10 % missing genotype data were removed. For the remaining markers, missing values were imputed by randomly assigning an allele based on the observed allele frequencies per marker. After filtering and imputation, a final dataset of 3333 markers scored across 200 accessions was used for further analysis.

2.6. GWAS procedure

Single-trait GWAS was conducted both within and across environments, depending on the BLUEs used as input. The analysis was performed using the statgenGWAS package in R (van Rossum and Kruijer, 2022). To account for population structure, a Van Raden kinship matrix was fitted and incorporated into the GWAS model. The effectiveness of population structure correction was evaluated by examining quantile-quantile (QQ) plots of expected versus observed P-values. To control the false discovery rate, multiple testing correction was applied following the approach of (Brzyski et al., 2017).

2.7. Candidate gene analysis

Genes surrounding significant markers were identified based on their annotations in the EL10.2 sugar beet genome (McGrath et al., 2023). The physical positions of the significant markers were provided by KWS SAAT SE & Co. KGaA. To define the genomic regions for investigation, a genome-wide linkage disequilibrium (LD) decay threshold of 501 Kb, as estimated by (Wang et al., 2024), was applied. Particular focus was given to candidate genes associated with chloroplast development and division, protein synthesis and degradation, protein and peptide transport, photosynthesis, and the synthesis and breakdown of the cell wall.

3. Results

3.1. Large phenotypic variability

The data analysis of the field trials involved fitting linear mixed

models to obtain Best Linear Unbiased Estimates (BLUEs) of genotypic means. Large phenotypic variability was observed for extractable leaf protein content (ELPC), extractable RuBisCO (ER), protein extractability (PE) and leaf yield (LY), as these traits had a coefficient of variation (CV %) from 8 % to 15 % (Table 2). The adjusted genotypic means for trial NL23 were distinctly different from the other three trials. Here, the adjusted means for LPC, ELPC, ER and RY were notably higher; with 28.41 % compared to 19.51–20.52 % (LPC), 13.35 % compared to 8.22 – 10.29 % (ELPC), 5.03 compared to 2.42–2.85 % (ER), and 1217.71 compared to 999.59–1074.16 (RY). In contrast, the values for SC were much lower as NL23 had a mean SC of 15.11 % compared to 17.60–18.26 % in the other three field trials. For protein extractability (PE), trial FR22 was 7–10 % lower than that of the other three trials.

3.2. High heritability for sugar content, root yield and leaf protein content

The broad-sense heritability was estimated within and across trials (Table 3). The highest heritability estimates were observed for traits measured on the beet root, with root yield (RY) and sugar content (SC) showing across trial estimates of 0.75 and 0.88, respectively. Among leaf-related traits, LPC and ER also exhibited high heritability across trials, 0.65 and 0.61, respectively. DM and ELPC revealed moderate heritability estimates of 0.51 and 0.48, while LY and PE showed lower heritability, at 0.27 and 0.26, respectively. The analysis of variance indicated that environment (E) had a significant effect ($P < 0.01$) on LPC, ELPC, ER, RY and SC, but not on DM, PE and LY. Genotype was significant for all traits, except PE and LY, where P values of 0.07 and 0.17 were obtained. A significant genotype-by-environment interaction was also observed for ER, RY, SC and PE ($P < 0.05$), but not for the other traits.

3.3. Trait correlations among beet and leaf protein components

Using the across trial BLUEs, correlation analysis was performed between the investigated traits. Additionally, we calculated sugar yield (SY*), total leaf protein (TLP*), protein yield (PY*) and RuBisCO yield (RubY*) using the across trial BLUEs of observed traits, and added them to the correlation analysis. Interestingly, a slight positive correlation was observed between sugar yield, and both protein and RuBisCO yield (Fig. 1), suggesting that protein yield can be selected for without compromising on sugar yield. As expected, a strong negative correlation (-0.76) was found between sugar content (SC) and root yield (RY) (Fig. 1), reflecting the typical trade-off in sugar beet breeding RY had a positive correlation (0.37) with LPC, which in turn had a negative correlation (-0.39) with SC, suggesting that increasing leaf protein may slightly reduce sugar concentration but no considerable effect on overall root yield. The leaf protein traits were also observed to correlate positively with each other. Extractable leaf protein content (ELPC) and protein extractability (PE) had a strong positive correlation (0.71), while ELPC was also positively correlated with LPC (0.55), and to a lesser extent with ER (0.39). Furthermore, the correlation between ER and LPC was strong (0.71). A moderate negative correlation was found between leaf yield (LY) and LPC (-0.35), indicating that higher leaf biomass may slightly dilute protein concentration (Fig. 1). Overall, these relationships highlight that improving leaf protein yield is feasible without major trade-offs for key sugar beet agronomic traits, offering opportunities for dual-purpose breeding.

Relationships between phenotypes were also investigated through principal component analysis (PCA), and visualized with a biplot over the first two dimensions (Fig. 2). The first two principal components explain 59.6 % of the variation. PC1 is largely influenced by the positive correlations between TLP*, PY* and RubY*, reflecting accessions with high total and extracted leaf production while variation in PC2 is dominated by LPC, ER, RY, and their negative correlation with SC and DM (supplementary, Table S1), highlighting the trade-off between sugar concentration and protein-related traits. Overall, the PCA results largely support the results of correlation analysis and emphasize two main breeding directions: (i) accessions combining high sugar content and leaf dry matter, and (ii) accessions with large roots and high protein yields.

The broad spread of accessions across the biplot confirms the presence of substantial phenotypic diversity, which provides valuable basis for selection. The top 10 accessions which contribute most to principal component 1 are highlighted in red (Fig. 2). Accessions on the left (towards the arrows of SC and DM) rank high in leaf dry matter and beet sugar content, while accessions on the right (towards the arrows of RY, SY, TLP and RubY) have larger beet roots and more protein in the leaves. Accession #093 may be especially interesting as it combines high SY with high RubY, suggesting potential for dual-purpose utilization (sugar and protein). Identifying such accessions demonstrate that it is feasible to select for enhanced protein yield without any negative effect on sugar yield, which is of direct agronomic and breeding relevance.

3.4. Identification of marker-trait associations (MTAs) via GWAS

Association mapping was performed to detect significant associations between genetic markers and variation in LPC, ELPC, ER, PE and LY. This was done in multiple analyses, using both the within- and across-trial BLUEs. The Manhattan plots for the association analyses are presented in supplementary Figure 1. A Van Raden kinship matrix proved to be adequate to correct for population structure, as there was minimal structure in the panel (supplementary Figure 2, Fig. 3). Across all analyses, a total of 182 marker-trait associations were identified as significant (FDR, $P < 0.05$) (Fig. 3). At times, the same marker was identified across separate analyses of trials, or shared among different traits. A total of 24 markers were identified twice, and 7 markers were identified as significant in three separate analyses. Of the markers that were identified as significant three separate times, three localize to the hotspot of significant marker-trait associations (MTAs) at the end of chromosome 8 (Fig. 3).

Using the list of significant markers from all analyses, important QTL regions were highlighted. Markers located within 10 cM of each other

Table 2
Phenotypic variation. Summary statistics of the investigated phenotypes (BLUEs) for each trial. LY = leaf yield per plant (g), DM = dry matter content (% FM), LPC = leaf protein content (% DM), ELPC = extractable leaf protein content (% DM), ER = extractable RuBisCO content (% DM), PE = protein extractability (% LPC), RY = root yield (dt/ha), SC = sugar content (% FM). LY was not determined in NL23 and FR23 trials (indicated as "-").

	NL22				NL23				FR22				FR23			
Trait	**Min**	**Mean**	**Max**	**CV**	**Min**	**Mean**	**Max**	**CV**	**Min**	**Mean**	**Max**	**CV**	**Min**	**Mean**	**Max**	**CV**
LY	12.66	20.15	31.93	15.73	-	-	-	-	7.47	16.14	24.13	15.87	-	-	-	-
DM	13.97	16.20	19.64	5.06	12.05	13.68	15.67	5.35	13.84	16.60	19.39	5.28	12.20	14.18	18.19	6.55
LPC	17.64	20.51	23.65	6.49	25.10	28.41	31.43	4.44	15.52	19.51	23.38	7.50	17.65	20.52	24.38	5.38
ELPC	7.09	9.94	12.90	11.32	9.95	13.35	16.16	8.97	5.61	8.22	12.37	13.19	6.93	10.29	12.61	9.57
ER	1.55	2.43	3.38	15.16	3.91	5.03	6.18	8.14	1.67	2.61	4.13	15.10	2.08	2.85	3.68	9.73
PE	31.05	48.46	64.34	11.05	36.96	47.11	55.39	8.16	29.59	40.70	51.42	10.86	36.64	50.15	59.29	8.32
RY	696.8	1011.6	1202.1	8.4	977.1	1217.7	1393.0	6.5	866.8	1074.2	1202.9	6.1	830.3	999.6	1117.9	5.3
SC	16.55	18.08	20.14	3.43	13.67	15.11	16.53	3.69	16.16	17.60	18.96	2.95	16.85	18.26	19.60	2.56

Table 3
Broad-sense heritability. Within and across trial broad sense heritability estimates. LY = leaf yield per plant (g), DM = dry matter content (% FM), LPC = leaf protein content (% DM), ELPC = extractable leaf protein content (% DM), ER = extractable RuBisCO content (% DM), PE = protein extractability (% LPC), RY = root yield (dt/ha), SC = sugar content (% FM).). LY was only determined in NL22 and FR22 trials.

Trait	Within trial heritability				Across trial variance components and heritability					
	NL22	NL23	FR22	FR23	G	E	Block	GEI	ε	H^2
LY	0.29	-	0.18	-	1.14	4.11	0.82	0.38	11.51	0.27
DM	0.19	0.54	0.35	0.39	0.19	0.27	1.85	0.05	1.36	0.51
LPC	0.41	0.52	0.36	0.63	0.65	16.87	0.20	0.17	2.50	0.65
ELPC	0.13	0.33	0.18	0.31	0.23	4.31	0.28	0.11	1.78	0.48
ER	0.41	0.46	0.43	0.31	0.04	1.39	0.02	0.02	0.16	0.61
PE	0.08	0.2	0.34	0.18	1.45	7.59	3.43	2.32	27.96	0.26
RY	0.66	0.63	0.79	0.67	2350.6	9004.0	747.6	750.9	4701.8	0.75
SC	0.72	0.85	0.85	0.87	0.18	2.15	0.05	0.02	0.15	0.88

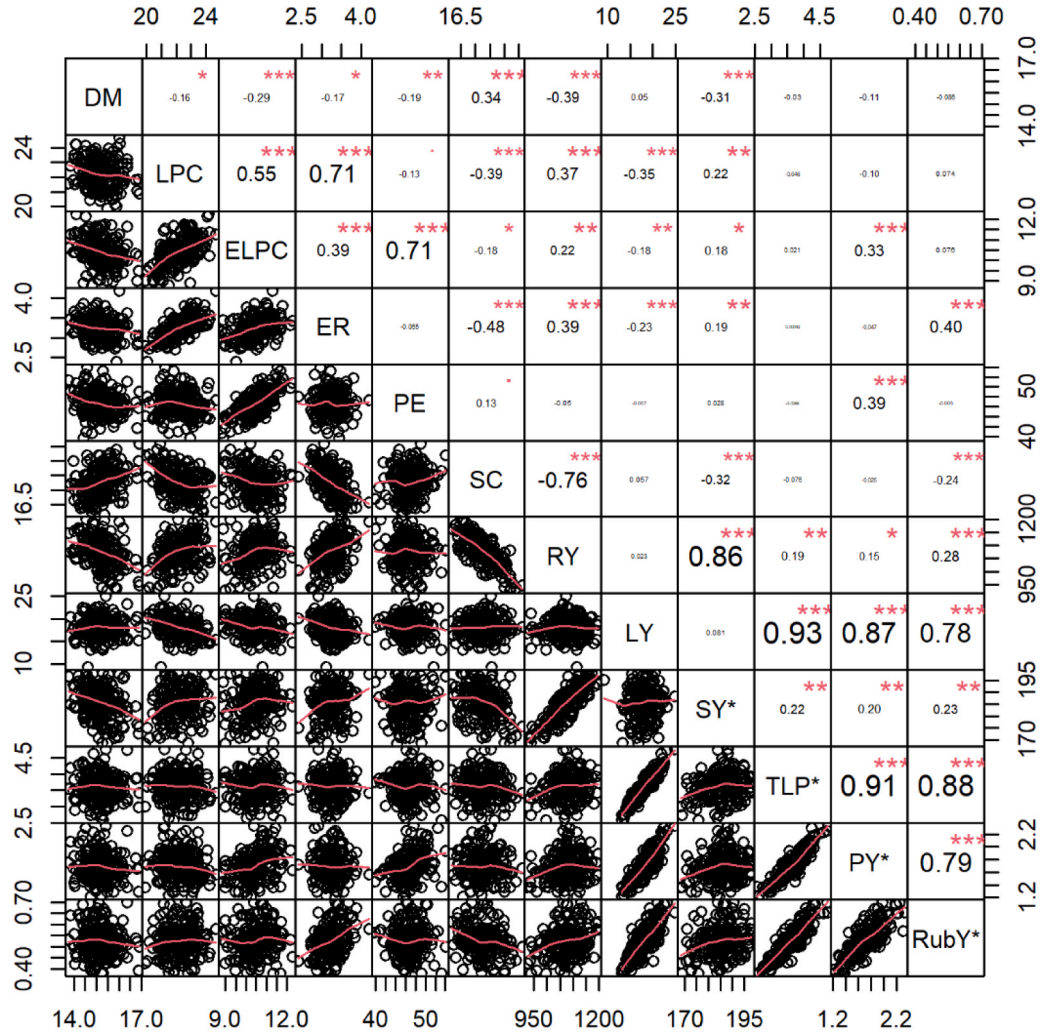


Fig. 1. Correlations between traits. Phenotypic correlation between traits, using the adjusted genotypic means obtained across trials. DM = Dry matter content (% FM), LPC = Leaf protein content (% DM), ELPC = Extractable leaf protein content (% DM), ER = Extractable RuBisCO (% DM), PE = Protein extractability (% LPC), SC = Sugar content (% FM), RY = Root yield (dt/ha), LY = Leaf yield (g DM per plant), SY* = Sugar yield (dt/ha), TLP* = Total leaf protein (dt/ha), PY* = Protein yield (dt/ha), RubY* = RuBisCO yield (dt/ha). *Variables are not BLUEs themselves, but rather calculated using BLUEs of other traits.

were considered to be part of the same QTL. This approach led to the identification of 12 QTLs for LPC, 12 for ELPC, 9 for ER, 9 for PE, and 6 for LY (Supplementary Table S2).

Several QTLs for multiple traits co-localized to the same genomic regions, suggesting that a common causal allele may underlie variation in multiple traits. Notable examples include the terminal region of chromosome 8, where QTL_LPC_8b, QTL_ELPC_8b and QTL_ER_8 overlap. Similarly, QTL_LPC_6b, QTL_ELPC_6a, and QTL_ER_6 cluster near the end of chromosome 6. Similarly, on chromosome 9, QTL_ELPC_9 and QTL_PE_9 overlap and also share common markers.

QTLs consistently identified across multiple trials provide stronger evidence of their validity, as they are repeatedly detected from independent phenotypic observations. Table 4 includes a column indicating the trials in which each QTL was detected. Notable examples include QTL_LPC_6c, QTL_ELPC_4a, QTL_ER_4b and QTL_LY_5b. Theses QTLs had a LOD score > 3 and they explained > 10 % variance of the

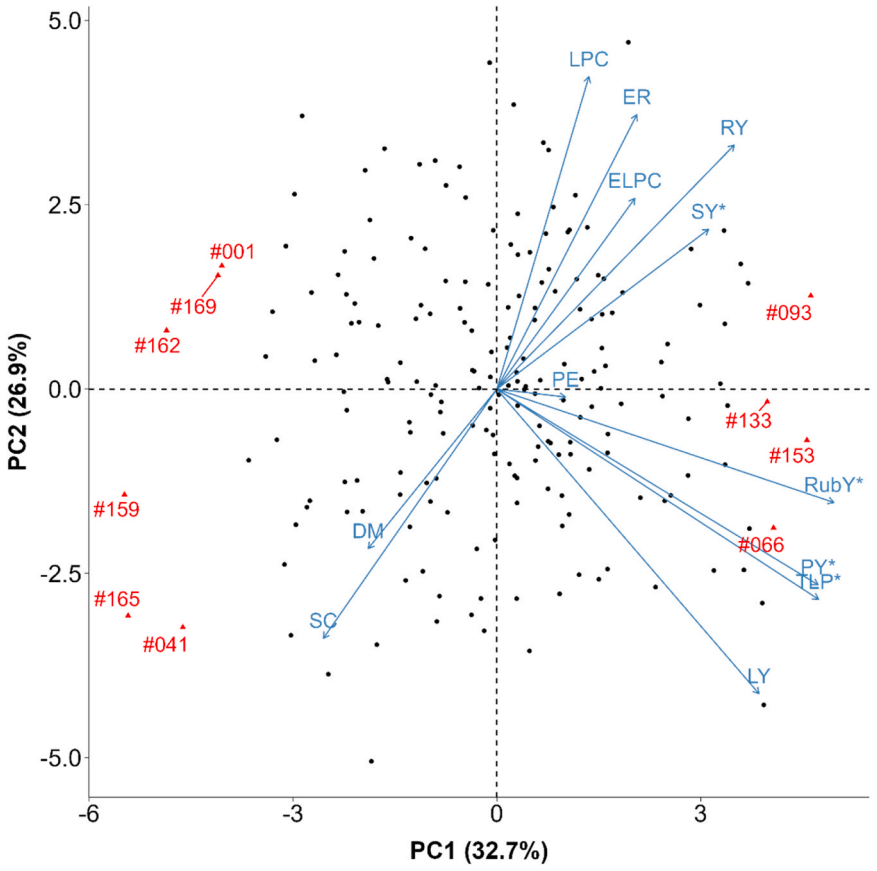


Fig. 2. Principal component analysis over phenotypic data. Principal component analysis over the investigated traits within a panel of 200 sugar beet accessions. The biplot shows the PCA loadings of the explanatory variables (arrows) and the scores of accessions (points). The top 10 contributing (PC1) accessions are highlighted in red.

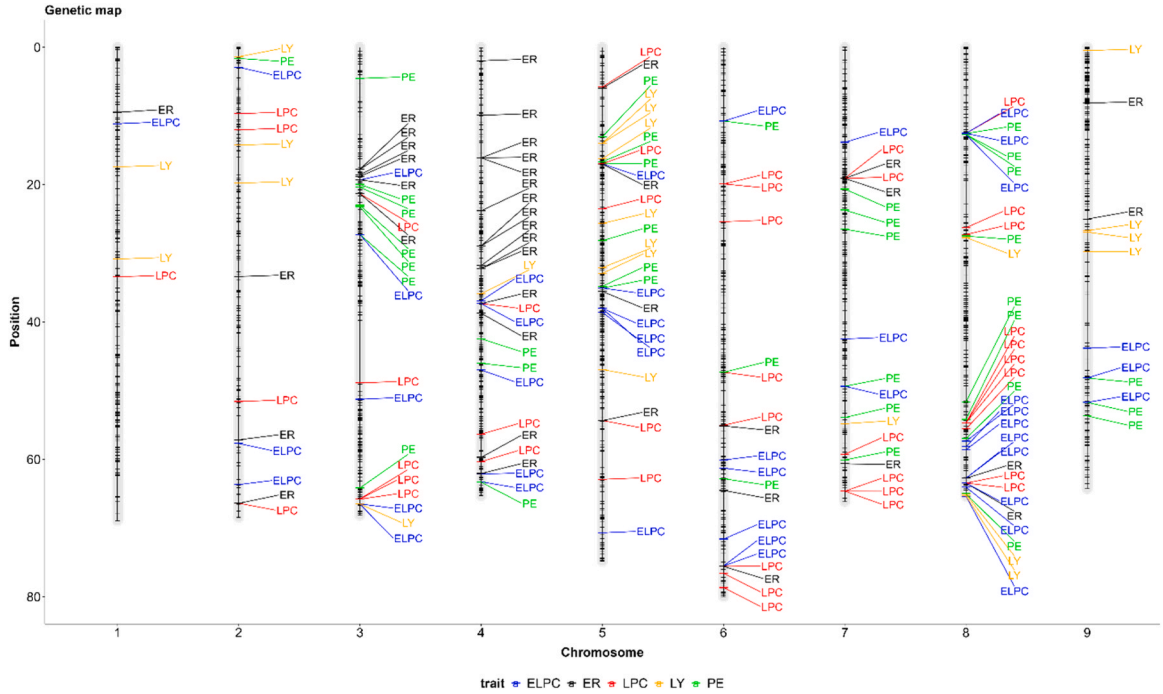


Fig. 3. Significant marker-trait associations. Genetic map of the nine chromosomes of sugar beet, showing the marker density and the locations of significant markers for leaf protein traits. LPC = Leaf protein content (% DM), ELPC = Extractable leaf protein content (% DM), ER = Extractable RuBisCO (% DM), PE = Protein extractability (% LPC), LY = Leaf yield (g DM per plant).

Table 4
QTL regions for leaf traits. Compiled QTLs for LPC, ELPC, ER, PE and LY. Association statistics refer to the most significant marker found within the QTL region. Chr = Chromosome, Hits = Number of significant markers, LOD = Association strength expressed as -log10(p-value), %Var = Percentage of variance explained by the marker, as determined within the analysis in the Trial column.

QTL name	Detected in trials	Chr	Position (cM)	Hits	Allele Freq	Effect	LOD	%Var	Trial
QTL_LPC_4	Across trials; NL22	4	56.33–60.34	2	0.32	−0.40	2.40	7.73	NL22
QTL_LPC_6b	NL22	6	47.32–55.04	2	0.24	0.55	3.16	12.63	NL22
QTL_LPC_6c	NL23; FR23	6	75.58–78.68	3	0.42	0.51	4.34	16.21	NL23
QTL_LPC_8b	Across trials; NL22; FR22; NL23	8	54.69–63.46	6	0.48	0.42	3.68	8.23	FR22
QTL_ELPC_2	NL22	2	57.7–63.67	2	0.07	−0.72	3.83	10.10	NL22
QTL_ELPC_4a	Across trials; FR22	4	36.92–37.33	2	0.23	−0.43	3.43	11.16	FR22
QTL_ELPC_4b	Across trials; FR22	4	62.13–63.27	2	0.23	−0.21	3.07	7.59	Across trials
QTL_ELPC_8b	Across trials; NL22; FR22; NL23; FR23	8	57.37–65.36	8	0.47	0.30	3.29	6.41	NL23
QTL_ER_4b	Across trials; FR22; FR23	4	16.12–23.82	4	0.48	−0.09	4.17	11.65	Across trials
QTL_ER_4c	Across trials; FR22; FR23	4	28.94–38.73	7	0.18	−0.16	3.70	9.46	FR22
QTL_ER_4d	NL22; FR22	4	59.72–62.07	2	0.39	−0.12	2.72	9.38	NL22
QTL_ER_8	FR23	8	62.74–63.46	2	0.41	0.08	3.35	8.54	FR23
QTL_PE_5a	NL22; NL23	5	13.04–16.98	3	0.13	−2.23	3.37	8.01	NL22
QTL_LY_5a	Across trials; FR22	5	14–16.23	3	0.32	−0.69	3.45	8.19	Across trials
QTL_LY_5b	Across trials; NL22	5	25.65–32.93	3	0.22	−1.22	3.47	12.53	NL22

associated phenotype.

Beyond their consistency across analyses, some QTL regions were detected only in a specific trial but they contained markers with substantial effects on the traits. For example, QTL_LPC_6b was detected in NL22 and explained 12.63 % variance in LPC, QTL_ELPC_2 was detected in NL22 and explained 10.1 % variance in ELPC, and QTL_ER_8 was detected in FR23 and explained 8.54 % variance in ER (Table 4).

3.5. Candidate genes

Out of the 182 marker-trait associations identified, 169 were intragenic, potentially highlighting candidate genes through direct association. Table 5 presents the potential candidate genes for leaf protein traits, with a full list provided in Supplementary Table S3. For LPC, notable candidates include *carbonic anhydrase 2* (CA2) and the *nitrate transporter NRT1.2* on chromosome 6, which are linked to photosynthesis and nitrate transport, respectively. Another candidate gene is amino acid export gene *GLUTAMINE DUMPER 3* (GDU3), which was consistently identified for multiple traits including LPC, ELPC and ER, suggesting that this is an important target for leaf protein traits. Other candidate genes for ELPC include genes involved in cell wall synthesis and degradation, such as CSLE1 and PAE6 (Table 5). Other notable candidates genes for ER include *Thioredoxin F-type* (TRXF) and *TIC20-II* on chromosome 4. TRXF plays a role in activating enzymes of the photosynthesis carbon cycle. *TIC20-II* is specific to photosynthetic preproteins and is known to be involved in the import of protein precursors into the chloroplast, For PE, a potential candidate gene includes TBL23 which is involved in cell wall modification. And finally for LY, a putative candidate gene related to plant growth (HPGT1) was identified.

4. Discussion

This study aimed to identify genotypes with high leaf protein extraction potential and explore the genetic basis of this trait to inform future breeding strategies. Across four field trials, we measured leaf protein content (LPC), extractable leaf protein content (ELPC), extractable RuBisCO (ER), and leaf yield (LY). Additionally, root yield (RY) and sugar content (SC) were assessed and compared to the leaf traits, to evaluate whether increased leaf protein extraction yield may compromise sugar yield. A genome-wide association study (GWAS) was conducted to identify key genetic markers associated with higher leaf protein extraction yields, providing insights into the genetic basis of this trait and potential targets for marker-assisted selection. Additionally, we examined genes located near significant markers and, based on gene annotations, identified potential candidate genes that may contribute to variation in leaf protein extraction yield.

The GWAS panel exhibited substantial phenotypic variation for the investigated leaf traits, as indicated by coefficients of variation (CV%) of 4.5–15 %. In NL23, the values for RY, LPC, ELPC and ER were notably higher compared to those of other trials. All four trails were conducted

Table 5
Candidate genes for leaf protein traits. Candidate genes found in linkage disequilibrium with significant marker-trait associations. Distance = distance in bp from marker to the candidate gene, when distance is 0 markers are intragenic.

QTL	Distance (bp)	Gene ID:	Gene acronym	Involved in / influences	Citation
QTL_LPC_4	0	LOC104891741	LHCA5, chloroplastic	Photosynthesis	(Ganeteg et al., 2004)
QTL_LPC_6b	33,933	LOC104897387	CA2	CO2 supply, photosynthesis	(DiMario et al., 2016; Okabe et al., 1984)
QTL_LPC_6c	42,137	LOC104897983	NRT1.2 / NPF4.6	Nitrate transporter	(Huang et al., 1999)
QTL_LPC_8b	370,436	LOC104902433	GDU3	Amino acid export	(Pratelli et al., 2010)
QTL_ELPC_4a	0	LOC130591297	PAE6	Cell wall modification	(de Souza et al., 2014)
QTL_ELPC_4b	22,407	LOC104891977	CSLE1	Cell wall modification; hemicellulose	(Richmond and Somerville, 2000)
QTL_ELPC_8b	370,436	LOC104902433	GDU3	Amino acid export	(Pratelli et al., 2010)
QTL_ER_4b	129,077	LOC104890206	TRXF	Redox regulation of chloroplastic carbon fixation enzymes	(Dai et al., 2007)
	169,035	LOC104890201	TIC20-II, chloroplastic	Protein import into chloroplasts	(Kikuchi et al., 2009)
QTL_ER_4c	0	LOC104890457	FTSHI 5, chloroplastic	Chloroplast development	(Lu et al., 2014)
QTL_ER_8	369,445	LOC104902433	GDU3	Amino acid export	(Pratelli et al., 2010)
QTL_PE_5a	3465	LOC104892185	TBL23	Cell wall modification	(Bischoff et al., 2010)
	5632	LOC104892252	PG	Cell wall modification	(Fry, 1995)
QTL_LY_5a	37,673	LOC104892203	HPGT1	Plant growth	(Ogawa-Ohnishi and Matsubayashi, 2015)

under the recommended management practices. However, differences in soil structure, composition and fertility between trial sites are plausible. In addition, environment factors such as temperature, precipitation and solar radiation likely contributed to variation in sugar beet growth across trials. In the present study, it was not possible to disentangle the specific impact of environmental conditions and management practices due limited available information. Nevertheless, these results emphasize the strong influence of environmental conditions and management practices on leaf protein yield, highlighting the need for future studies to systematically evaluate their contributions.

4.1. Estimating the genetic basis of leaf protein traits through broad-sense heritability (H^2)

The heritability of root yield and sugar content was higher than that of leaf protein traits, which is expected given that the germplasm originates from an active sugar beet breeding program. Since sugar yield is the primary breeding target, the germplasm used in this study was likely chosen to include a broad range of genetic variation for root yield and sugar content, contributing to higher heritability estimates. A low to moderate heritability estimates were obtained for LPC, ELPC and ER for within trail analysis. Given that the method of extraction in this study aims to separate the protein fractions based on their solubility, our results may be compared to investigations into the heritability of soluble leaf protein in alfalfa. In studies from Gutek et al. (1976); Hansen et al. (1992), varying heritability estimates were obtained ranging from 0.03 to 0.46. In this study, we also found varying heritability estimates (0.13 – 0.46) for soluble protein fractions, when estimating the heritability within each trial. This likely reflects the difficulty of the measurement, where small laboratory errors can quickly influence the result. Additionally, variation in environmental conditions and plant age may contribute to the observed range in heritability estimates. Still, the heritability was moderate in the across trial analysis, suggesting that even after adjusting for environmental variation, a substantial proportion of the phenotypic variation is genetically determined and can be further improved through targeted selective breeding. The percentage of extractable protein out of total protein, as expressed by PE, had a low heritability. In the PE model, the residual variance was high, indicating that phenotypic values are difficult to explain using standard input factors typically applied in breeding trials. This is likely due to the influence of numerous underlying factors, such as cell wall composition, polyphenol oxidase (PPO) and protease activity, that affect PE. Additionally, the complexity of the PE measurement itself, being a derived trait based on multiple component measurements, further contributes to this unexplained variability. Taken together our results indicate that direct selection for PE offers limited potential to improve leaf protein content, but indirect selection through component traits such as LPC, ELPC and ER remains promising.

4.2. Correlations between phenotypes + PCA

We observed stronger correlation between LPC and ER, compared to LPC and ELPC, which was not expected as both LPC and ELPC are derived from measuring the total nitrogen content of the sample, while ER derived from size-exclusion chromatography analysis which targets RuBisCO based on its larger size. Furthermore, while ELPC and ER are both quantifications of the extractable protein fractions in the leaf juice, their correlation was only 0.39. Both findings may be explained by the accumulation of small non-protein nitrogen (NPN) compounds in the juice supernatant that is measured for ELPC. Due to the small size and solubility of NPN compounds, these will accumulate in the juice fraction (Nynäs et al., 2024), and cause a greater distortion of protein measurements based on total nitrogen, compared to when measuring dried leaves (LPC).

Negative correlation between root yield and sugar content is a known challenge for beet breeders. When it comes to leaf protein, a positive correlation was observed between LPC and RY, indicating that sugar beets with larger beet roots also have higher protein contents in the leaves. This higher total protein content also translates to higher contents of extractable protein (ELPC & ER), selections to increase RY and LPC may therefore also enhance leaf protein extraction yield indirectly.

According to Hoffmann and Kenter (2018), increases in sugar yield over the years have come at the expense of leaf dry matter and cell wall compounds in the storage root. Our results agree with this finding, as leaf dry matter and sugar yield were negatively correlated. Our results show a small negative correlation between leaf dry matter and protein content, which may indicate that the loss in leaf dry matter did not come from a decrease in leaf protein. Overall, there is a slight positive correlation between sugar yield and protein, as well as RuBisCO yield, indicating that targeted breeding for increased leaf protein yield is possible without interfering the yield of the main harvested product of sugar beet. It has to be said though, sugar yield in this study was calculated from the beet root's sugar content, and it's yield per hectare. It is therefore only a measure of the total available sugar, and not an actualized measure of sugar yield after extraction. Extraction of sugar from the beet root is hampered by the presence of amino N compounds, and low amino N is therefore an important quality criteria in sugar beet breeding. However, amino N compounds are also essential for plant growth and development, including root growth, suggesting a potential trade-off between root yield and root quality. In research from Mäck et al. (2007), the allocation of nitrogen compounds in sugar beet organs was studied using two genotypes that contrast in their amino N concentration in the beet. Here, the high amino N beet variety was also found to have significantly higher amino N contents in the mature leaves. The total soluble protein content in the mature leaves was also higher in the high amino N genotype, but this was not significant. Ultimately, Mäck et al. (2007) conclude that differences between genotypes in the amino N concentration of the root is more likely to result from differences in glutamine synthetase (GS) activity in the beet, rather than in the leaves. GS is an enzyme active in ammonia assimilation as it uses glutamate and ammonia to make glutamine (Miflin & Lea, (1980)), which is the main amino N constituent in the beet (Mäck, (1988)). Glutamate is the main amino N constituent in the leaves, where it is loaded from the cytosol into the phloem and transported to the beet (Lohaus et al., 1994). As LPC is based on measurements of total nitrogen, accessions with high LPC are likely to contain higher levels of glutamate and other amino acids in the leaves. When translocated to the roots, this may benefit root biomass production, but it could also lead to reduced root quality for sugar extraction. In the present study, it was not possible to test the potential trade-off between increased leaf protein yield and the beet root quality, as the data on beet amino Nitrogen was not available. This limitation highlights an important area for future research, where integrated assessment of both leaf protein and beet root quality traits will be crucial to fully understand the implications of selecting high leaf protein in sugar beet.

4.3. Association mapping

The GWAS identified 182 marker-trait associations, consolidated into 48 QTLs across the genome. Several QTLs were consistently detected across multiple trials, and some contains markers explaining 10 % of the phenotypic variance, highlighting their potential for molecular breeding of leaf protein and related traits. Most significant markers (92.8 %) were intragenic, But as synonymous mutations may also be intragenic that may not alter gene function, we also considered neighboring genes in linkage disequilibrium (LD) to identify the most likely candidate genes.

Near the end of chromosome 8, overlapping QTLs for LPC, ELPC and ER were identified, with GLUTAMINE DUMPER 3 (GDU3) as a potential candidate gene. GDUs are known to mediate nonselective amino acid export from leaves (Pratelli et al., 2010), and variation in this process

may affect amino acid availability for protein synthesis. These QTLs may be valuable for breeding, as reducing amino acid export from leaves could enhance protein content. Future studies could also explore potential effects on root yield and quality, as excessive amino N translocation may negatively impact root quality.

Other candidate genes for LPC include those involved in photosynthesis, such as LHCA5 and carbonic anhydrase 2, as well as genes related to the transport of nitrate. Notably, the low-affinity nitrate transporter NRT1.2/NPF4.6 was identified within QTL_LPC_6c, with particular significant markers in the NL23 trial, which showed higher LPC and RY likely due to improved soil composition and fertility. As NRT1.2/NPF4.6 is active at higher nitrate concentrations (Huang et al., 1999; Dreyer and Michard, 2019), the stronger QTL signal in NL23 may reflect nitrate rather than true protein content. Because nitrates contribute to non-protein nitrogen, this QTL may be less useful for selecting varieties with genuinely high leaf protein content.

A candidate gene, carbonic anhydrase 2 (CA2) within QTL_LPC_6b is known to be a relatively abundant protein, comprising 1–2 % of the soluble protein in spinach (Okabe et al., 1984). As zinc metalloenzymes, CAs catalyze the interconversion of CO2 and HCO3-, supplying CO2 to the chloroplasts for photosynthesis. Interestingly, Arabidopsis CA mutants maintained photosynthesis but exhibited reduced amino acid levels, suggesting a key role for CAs in amino acid biosynthesis (DiMario et al., 2016).

For ER, gene involved in transport of protein precursors into the chloroplast; TIC20-II was found within QTL_ER_4b. In Arabidopsis, TIC20 mutants show impaired import of photosynthetic proteins into chloroplasts (Kikuchi et al., 2009), suggesting these genes may influence RuBisCO content and variation in ER. Markers in QTL_ER_4b were highly significant, explaining up to 11.6 % of the phenotypic variance, and this region did not overlap with QTLs for LPC or ELPC, indicating its potential for selecting RuBisCO content specifically.

For ELPC and PE, multiple QTLs contained markers in coding regions or in LD with genes related to cell wall metabolism, including PAE6, CSLE1 and TBL23, consistent with previous reports that cell wall composition can limits leaf protein extraction yield (Kleuter et al., 2024; Sari et al., 2015; Tamayo Tenorio et al., 2018). Enzymatic degradation of the cell wall during the extraction process has been shown to increase leaf protein extraction yield in sugar beet. For LY, candidate genes related to plant growth was identified, including HPGT1 at a consistent QTL on chromosome 5, Arabidopsis plants with loss of function mutants of HPGT1 together with HPGT2 and HPGT3, showed several growt defects including small leaves, suggesting that HPGT1 is an important gene for leaf yield in sugar beet (Ogawa-Ohnishi and Matsubayashi, 2015).

Overall, the candidate genes co-localizing with marker-trait associations for traits related to leaf protein extraction yield provide valuable insights into the genetic basis of trait variation. Further functional studies in sugar beet will be essential to validate their roles in leaf protein content and extractability.

5. Conclusion

Selective breeding could increase the leaf protein extraction yield from sugar beet leaves. Breeding for total leaf protein content will be more straightforward than breeding for protein extractability, as this trait has a lower heritability and is more difficult to measure. No negative relationships were observed between leaf protein extraction yield and root sugar yield, indicating that dual purpose breeding is possible without strong trade-offs for the sugar and root yield; the economic traits. The genetic architecture of leaf protein extraction yield is complex, as up to 48 QTLs related to LPC, ELPC, ER, PE or LY were identified. Several large effect marker-trait associations were identified, which explained more than 10 % of the phenotypic variance of the traits, notably those located in QTL_LPC_6b and 6c, QTL_ELPC_2, QTL_ELPC_4a, QTL_ER_4b and QTL_LY_5b. Within these QTLs, a number of candidate genes were detected, notably those related to photosynthetic processes, cell wall composition and plasticity, amino acid transport, and protein import into the chloroplast. The identified QTLs and candidate genes provide breeding tools to improve leaf protein yield in sugar beet. Our results support the sustainable valorization of sugar beet side-streams. Further research into the QTL regions, through fine mapping or gene functional studies would be a logic follow up step in order to further validate our results.

CRediT authorship contribution statement

Hugo Rijken: Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Aamir Saleem:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation. **Christian B. Hirschmann:** Writing – review & editing, Resources, Formal analysis. **Trindade Luisa:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Dieter Hackenberg:** Writing – review & editing, Resources, Formal analysis, Data curation. **Marieke E. Bruins:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT (OpenAI, free version) to improve the language and clarity of the written text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2025.110215.

Data availability

Data will be made available on request.

References

Akyuz, A., Ersus, S., 2021. Optimization of enzyme assisted extraction of protein from the sugar beet (Beta vulgaris L.) leaves for alternative plant protein concentrate production. Food Chem. 335, 127673. https://doi.org/10.1016/j.foodchem.2020.127673.

Barbeau, W.E., Kinsella, J.E., 1988. Ribulose bisphosphate carboxylase/oxygenase (rubisco) from green leaves-potential as a food protein. Food Rev. Int. 4 (1), 93–127. https://doi.org/10.1080/87559128809540823.

Bates, D., Machler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Biazzi, E., Nazzicari, N., Pecetti, L., Brummer, E.C., Palmonari, A., Tava, A., Annicchiarico, P., 2017. Genome-wide association mapping and genomic selection for alfalfa (medicago sativa) forage quality traits. PLOS One 12 (1), e0169234. https://doi.org/10.1371/journal.pone.0169234.

Bischoff, V., Nita, S., Neumetzler, L., Schindelasch, D., Urbain, A., Eshed, R., Persson, S., Delmer, D., Scheible, W.R., 2010. TRICHOME BIREFRINGENCE and its homolog AT5G01360 encode plant-specific DUF231 proteins required for cellulose biosynthesis in Arabidopsis. Plant Physiol. 153 (2), 590–602. https://doi.org/10.1104/pp.110.153320.

Brzyski, D., Peterson, C.B., Sobczyk, P., Candes, E.J., Bogdan, M., Sabatti, C., 2017. Controlling the rate of GWAS false discoveries. Genetics 205 (1), 61–75. https://doi.org/10.1534/genetics.116.193987.

Dai, S., Friemann, R., Glauser, D.A., Bourquin, F., Manieri, W., Schurmann, P., Eklund, H., 2007. Structural snapshots along the reaction pathway of ferredoxin-thioredoxin reductase. Nature 448 (7149), 92–96. https://doi.org/10.1038/nature05937.

DiMario, R.J., Quebedeaux, J.C., Longstreth, D.J., Dassanayake, M., Hartman, M.M., Moroney, J.V., 2016. The cytoplasmic carbonic anhydrases betaCA2 and betaCA4 are required for optimal plant growth at low CO2. Plant Physiol. 171 (1), 280–293. https://doi.org/10.1104/pp.15.01990.

Dreyer, I., Michard, E., 2019. High- and low-affinity transport in plants from a thermodynamic point of view. Front. Plant Sci. 10, 1797. https://doi.org/10.3389/fpls.2019.01797.

Ducrocq, M., Morel, M.H., Anton, M., Micard, V., Guyot, S., Beaumal, V., Sole-Jamault, V., Boire, A., 2022. Biochemical and physical-chemical characterisation of leaf proteins extracted from Cichorium endivia leaves. Food Chem. 381, 132254. https://doi.org/10.1016/j.foodchem.2022.132254.

Fiorentini, R., Galoppini, C., 1983. The proteins from leaves. Plant Foods Hum. Nutr. 32, 335–350. https://doi.org/10.1007/BF01091193.

Fry, S., C., 1995. Polysaccharide-modifying enzymes in the plant cell wall. Annu. Rev. Plant Biol. 46, 497–520. https://doi.org/10.1146/annurev.pp.46.060195.002433.

Ganeteg, U., Klimmek, F., Jansson, S., 2004. Lhca5 - an LHC-type protein associated with photosystem I. Plant Mol. Biol. 54, 641–651. https://doi.org/10.1023/b:plan.0000040813.05224.94.

Gutek, L.H., Goplen, B.P., Howarth, R.E., 1976. Heritability of soluble proteins in Alfalfa1. Crop Sci. 16 (2), 199–201. https://doi.org/10.2135/cropsci1976.0011183X001600020008x.

Hansen, J.L., Viands, D.R., Steffens, J.C., Sniffen, C.J., 1992. Heritability and improvement of protein and nitrogen concentrations in wilted Alfalfa forage. Crop Sci. 32 (4), 879–883. https://doi.org/10.2135/cropsci1992.0011183X003200040009x.

Hergert, G.W., 2011. Sugar beet fertilization. Sugar Tech. 12 (3-4), 256–266. https://doi.org/10.1007/s12355-010-0037-1.

Hoffmann, C.M., Kenter, C., 2018. Yield potential of sugar beet - have we hit the ceiling? Front Plant Sci. 9, 289. https://doi.org/10.3389/fpls.2018.00289.

Huang, N., Liu, K., Lo, H., Tsay, Y., 1999. Cloning and functional characterization of an arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake. Plant Cell 11, 1381–1392. https://doi.org/10.1105/tpc.11.8.1381.

Kassambara, A., Mundt, F., 2020. Factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. https://CRAN.R-project.org/package=factoextra.

Kikuchi, S., Oishi, M., Hirabayashi, Y., Lee, D.W., Hwang, I., Nakai, M., 2009. A 1-megadalton translocation complex containing Tic20 and Tic21 mediates chloroplast protein import at the inner envelope membrane. Plant Cell 21 (6), 1781–1797. https://doi.org/10.1105/tpc.108.063552.

Kiskini, A., Vissers, A., Vincken, J.P., Gruppen, H., Wierenga, P.A., 2016. Effect of plant age on the quantity and quality of proteins extracted from sugar beet (Beta vulgaris L.) Leaves. J. Agric. Food Chem. 64 (44), 8305–8314. https://doi.org/10.1021/acs.jafc.6b03095.

Kleuter, M., Yu, Y., Pancaldi, F., Nagtzaam, M., van der Goot, A.J., Trindade, L.M., 2024. Cell wall as a barrier for protein extraction from tomato leaves: a biochemical study. Plant Physiol. Biochem. 208, 108495. https://doi.org/10.1016/j.plaphy.2024.108495.

Lammens, T.M., Franssen, M.C.R., Scott, E.L., Sanders, J.P.M., 2012. Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. Biomass Bioenergy 44, 168–181. https://doi.org/10.1016/j.biombioe.2012.04.021.

Lin, S., Medina, C.A., Patel, S.B., Xu, Z., Zanton, G.I., Combs, D., Wang, G., Shewmaker, G., Fransen, S., Llewellyn, D., Norberg, S., Yu, L.-X., 2025. Identification of genetic loci associated with protein and fiber digestibility in alfalfa (Medicago sativa L. Crop Sci. 65, e70004. https://doi.org/10.1002/csc2.70004.

Lohaus, G., Burba, M., Heldt, H.W., 1994. Comparison of the contents of sucrose and amino acids in leaves, phloem sap and taproots of high and low sugar-producing hybrids of sugar beet (*Beta vulgaris* L.). J. Exp. Bot. 45, 1097–1101. https://doi.org/10.1093/jxb/45.8.1097.

Lu, X., Zhang, D., Li, S., Su, Y., Liang, Q., et al., 2014. FtsHi4 is Essential for embryogenesis due to its influence on Chloroplast development in arabidopsis. PLoS ONE 9 (6), e99741. https://doi.org/10.1371/journal.pone.0099741.

Mäck, G., Hoffmann, C.M., Märländer, B., 2007. Nitrogen compounds in organs of two sugar beet genotypes (Beta vulgaris L.) during the season. Field Crops Res. 102 (3), 210–218. https://doi.org/10.1016/j.fcr.2007.04.001.

Martin, A.H., Castellani, O., de Jong, G.A., Bovetto, L., Schmitt, C., 2019. Comparison of the functional properties of RuBisCO protein isolate extracted from sugar beet leaves with commercial whey protein and soy protein isolates. J. Sci. Food Agric. 99 (4), 1568–1576. https://doi.org/10.1002/jsfa.9335.

Martin, A.H., Nieuwland, M., de Jong, G.A., 2014. Characterization of heat-set gels from RuBisCO in comparison to those from other proteins. J. Agric. Food Chem. 62 (44), 10783–10791. https://doi.org/10.1021/jf502905g.

McGrath, J.M., Funk, A., Galewski, P., Ou, S., Townsend, B., Davenport, K., Daligault, H., Johnson, S., Lee, J., Hastie, A., Darracq, A., Willems, G., Barnes, S., Liachko, I., Sullivan, S., Koren, S., Phillippy, A., Wang, J., Liu, T., Dorn, K.M., 2023. A contiguous de novo genome assembly of sugar beet EL10 (Beta vulgaris L.). DNA Res. 30 (1). https://doi.org/10.1093/dnares/dsac033.

Montes, C.M., Fox, C., Sanz-Saez, A., Serbin, S.P., Kumagai, E., Krause, M.D., Xavier, A., Specht, J.E., Beavis, W.D., Bernacchi, C.J., Diers, B.W., Ainsworth, E.A., 2022. High-throughput characterization, correlation, and mapping of leaf photosynthetic and functional traits in the soybean (Glycine max) nested association mapping population. Genetics 221 (2), iyac065. https://doi.org/10.1093/genetics/iyac065.

Nynäs, A.-L., Berndtsson, E., Newson, W.R., Hovmalm, H.P., Johansson, E., 2024. Protein fractionation of leafy green biomass at the pilot scale: partitioning and type of nitrogen in the fractions and their usefulness for food and feed. ACS Food Sci. Technol. 4 (1), 126–138. https://doi.org/10.1021/acsfoodscitech.3c00426.

Ogawa-Ohnishi, M., Matsubayashi, Y., 2015. Identification of three potent hydroxyproline O-galactosyltransferases in Arabidopsis. Plant J. 81 (5), 736–746. https://doi.org/10.1111/tpj.12764.

Okabe, K., Yang, S., Tsuzuki, M., Miyachi, S., 1984. Carbonic anhydrase: its content in spinach leaves and its taxonomic diversity studied with anti-spinach leaf carbonic anhydrase antibody. Plant Sci. Lett. 33, 145–153. https://doi.org/10.1016/0304-4211(84)90004-X.

Pérez-Vila, S., Acevedo, F., Brodkorb, A., Dias, N.B., Fenelon, M.A., O'Mahony, J.A., Gómez-Mascaraque, L.G., 2025. Quinoa leaves as a source of soluble proteins: extraction and proteomic characterization from four different varieties of Chenopodium quinoa. Food Chem. 468, 142411. https://doi.org/10.1016/j.foodchem.2024.142411.

Peterson, B.G., Carl, P., 2024. Performance analytics: econometric tools for performance and risk analysis. R. Package Version 2 0. ⟨https://github.com/braverock/performanceanalytics⟩, 8-1.

Pratelli, R., Voll, L.M., Horst, R.J., Frommer, W.B., Pilot, G., 2010. Stimulation of nonselective amino acid export by glutamine dumper proteins. Plant Physiol. 152 (2), 762–773. https://doi.org/10.1104/pp.109.151746.

Richmond, T., A., Somerville, C., R., 2000. The cellulose synthase superfamily. Plant Physiol. 124, 495–498. https://doi.org/10.1104/pp.124.2.495.

Rijken, H., Oertel, H., F., Hirschmann, C., B., Tossens, A., Hackenberg, D., Vegas, J., Bruins, M.E., Trindade, L.M., 2025. High-throughput methods for measuring protein extractability in sugar beet (Beta vulgaris L.) leaves. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.70074.

Rodríguez-Álvarez, M.X., Boer, M.P., Fred, A., Eilers, P.H.C., 2018. Correcting for spatial heterogeneity in plant breeding experiments with P-splines. Spat. Stat. 23, 52–71. https://doi.org/10.1016/j.spasta.2017.10.003.

van Rossum, B.J., Kruijer, W., 2022. . R package version 1.0.9. statgenGWAS: Genome Wide Association Studies⟨https://CRAN.R-project.org/package=statgenGWAS⟩.

Sari, Y.W., Syafitri, U., Sanders, J.P.M., Bruins, M.E., 2015. How biomass composition determines protein extractability. Ind. Crops Prod. 70, 125–133. https://doi.org/10.1016/j.indcrop.2015.03.020.

de Souza, A., Hull, P.A., Gille, S., Pauly, M., 2014. Identification and functional characterization of the distinct plant pectin esterases PAE8 and PAE9 and their deletion mutants. Planta 240 (5), 1123–1138. https://doi.org/10.1007/s00425-014-2139-6.

Starke, P., Hoffmann, C., M., 2014. Yield parameters of Beta beets as a basis to estimate the biogas yield. Sugar Ind. 139, 169–176. https://doi.org/10.36961/si15365.

Tamayo Tenorio, A., Gieteling, J., de Jong, G.A.H., Boom, R.M., van der Goot, A.J., 2016. Recovery of protein from green leaves: overview of crucial steps for utilisation. Food Chem. 203, 402–408. https://doi.org/10.1016/j.foodchem.2016.02.092.

Tamayo Tenorio, A., Kyriakopoulou, K.E., Suarez-Garcia, E., van den Berg, C., van der Goot, A.J., 2018. Understanding differences in protein fractionation from conventional crops, and herbaceous and aquatic biomass - consequences for industrial use. Trends Food Sci. Technol. 71, 235–245. https://doi.org/10.1016/j.tifs.2017.11.010.

Wang, L., Song, B., Ishfaq, M., Zhao, X., 2025. Optimization of nitrogen fertilizer application enhanced sugar beet productivity and socio-ecological benefits in china: a meta-analysis. Soil Tillage Res. 251, 106547. https://doi.org/10.1016/j.still.2025.106547.

Wang, S., Yue, Z., Yu, C., Wang, R., Sui, Y., Hou, Y., Zhao, Y., Zhao, L., Chen, C., Yang, Z., Shao, K., 2024. Genome-wide association study identifies the genetic basis of key agronomic traits in 207 sugar beet accessions. Hortic. Res. 11 (10), uhae230. https://doi.org/10.1093/hr/uhae230.

Wurschum, T., Maurer, H.P., Kraft, T., Janssen, G., Nilsson, C., Reif, J. aC., 2011. Genome-wide association mapping of agronomic traits in sugar beet. Theor. Appl. Genet. 123 (7), 1121–1131. https://doi.org/10.1007/s00122-011-1653-1.