Euphytica (2024) 220:143
https://doi.org/10.1007/s10681-024-03400-8

RESEARCH

A genotype–phenotype approach to discriminate Central European spelt landraces from modern wheat-spelt intercrosses in the Swiss context

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Received: 18 April 2024 / Accepted: 31 July 2024

Abstract Marketed as a healthier alternative to bread wheat, spelt (*Triticum aestivum* ssp. *spelta)* is in increasing demand from consumers and bakers in Switzerland. The Swiss spelt landscape is currently dominated by two varieties—*Ostro* and *Oberkulmer Rotkorn*—considered as references for Swiss "typical" spelt. However, these two varieties are rather old and deliver low yields; the market therefore needs improved spelt varieties with higher agronomic potential while keeping the essential attributes of typical spelt. As spelt and winter wheat can be interbred, modern spelt varieties often result from crosses between the two. It has therefore become increasingly difficult to distinguish between typical spelt and modern wheat-spelt intercrosses. This project aims to clarify the phenotypic distinction between typical spelt and modern wheat-spelt intercrosses in the Swiss context. To do this, we performed field trials with 50 spelt varieties, including typical and modern cultivars from Switzerland and Central Europe. We measured agronomic, rheological and nutritional parameters of each variety. In addition, these cultivars were genotyped using a 25 K Illumina Wheat SNP array. This allowed us to identify which phenotypic parameters were associated with genetic proximity to typical spelt. Swiss typical spelt varieties were characterized by highly extensible doughs, later phenology, low harvest index, high thousand kernel weights, and lower Zeleny/protein ratio. By linking phenotypic characteristics, easily measurable in fields, to the underlying genetic information of each variety, these results will help to better classify spelt varieties

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Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10681-024-03400-8.

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in Switzerland and pave the way for more clarity and transparency on the Swiss spelt market.

Keywords Wheat-spelt intercrosses · Spelt classification · Dough elongation · Protein content · Phenotypic characters

Introduction

In Europe, only a few arable crop species represent a large proportion of cultivated lands (Oehen and Moschitz 2018): notably, bread wheat (*Triticum aestivum* ssp. *aestivum)*, maize (*Zea mays*), and barley (*Hordeum vulgare*) accounted for up to 86% of the cereals produced in the EU in 2022 (European Commission 2023). Yet impending climatic changes and extreme weather events, combined with the loss of biodiversity, make the current arable crop production system vulnerable to stresses (Allan et al. 2021; Congreves et al. 2016; Philip et al. 2020). In the face of growing climatic variability, increasing the portfolio of cultivated crops could improve the resilience of the production system by serving as insurance against the risks of extreme events, such as drought, heat, or heavy rainfall (Yachi and Loreau 1999). Indeed, if the conditions during the growing season are particularly bad for one crop species, having another set of crops on a significant percentage of the land might limit yield loss and, consequently, revenue loss (Auffhammer and Carleton 2018).

In this context, currently underutilized cereal crop plants could play an important role. These include, among others, rye (*Secale cereale*), oat (*Avena sativa*), emmer (*Triticum dicoccum*), einkorn (*Triticum monococcum*), and spelt (*Triticum aestivum* ssp. *spelta*). While spelt is the second most important cereal used in breadmaking after bread wheat (Oehen and Moschitz 2018), it only represents 6.4% of the total grain production area for human nutrition in Switzerland (Federal Statistical Office), making spelt a niche product. In the past decade, however, there has been an increase in market demand from consumers in Europe and in Switzerland (Alvarez 2021; Schober et al. 2006; Tóth et al. 2022). This increase in spelt demand can partially be attributed to the creation of the brand PureSpelt through the interest group "IG Dinkel". The group preserves the genetic identity of spelt and promotes the production of spelt in the country (Agri Hebdo 2016; PurEpeautre—La Céréale Précieuse). Currently, only two spelt varieties—*Ostro* and *Oberkulmer Rotkorn*—can be marketed under the brand name UrDinkel (meaning "PureSpelt") and are considered as references for "typical" spelt in Western Europe (www.urdinkel.ch, Wiwart et al. 2017). The term "typical" spelt in this manuscript describes a spelt genotype that has not been crossed with modern elite cultivars to improve agronomic performance. *Oberkulmer Rotkorn* and *Ostro* are rather old varieties—*Oberkulmer Rotkorn* was registered in 1948, and *Ostro* in 1978—and characterized by low yield, high susceptibilities to common diseases, and lodging (DSP—Delley Samen und Pflanzen AG, 2021; Longin and Würschum 2014). All the mentioned constraints represent obstacles to the larger adoption of spelt as a crop in Switzerland. To alleviate these limitations, there is a need to find new varieties of spelt that have better agronomic performances, while remaining similar to the traditional, typical Swiss spelt varieties in terms of grain quality and baking characteristics (Koenig et al. 2015; Tóth et al. 2022).

Spelt being a close relative of wheat, the two species can be interbred and crosses can be made easily (Abrouk et al. 2021; Wiwart et al. 2015; Xie et al. 2015). Such crosses between winter wheat and spelt have been regularly exploited by breeders to improve the performance of spelt, for instance, to reduce its susceptibility to lodging or to increase its threshability (Schmid and Winzeler 1990; Siedler et al. 1994; Wiwart et al. 2015). In central Europe, spelt varieties are now divided into two main groups based on their genetic proximity to common wheat: one group consists of "old" or "typical" spelt landraces—such as *Ostro* and *Oberkulmer Rotkorn*—while the second one consists of "modern" varieties that have been artificially crossed with modern wheat a varying number of times (Koenig et al. 2015; Müller et al. 2018). This has made it challenging to distinguish "typical" spelt from "modern" varieties on the Swiss market, leading to confusions among the processors and consumers (Koenig et al. 2015; Wiwart et al. 2017). For transparency reasons towards consumers, it is therefore important to better characterize the phenotypical specificities of Swiss typical spelt varieties in contrast to Swiss modern spelt varieties. Our research questions are, therefore, the following: firstly, what are the agronomic, morphological, nutritional, and baking characteristics of various spelt varieties ranging from modern wheat-type to typical spelt, and how do these traits correlate with genetic distances from bread wheat? Secondly, is it possible to identify some key phenotypic traits to discriminate between the two spelt types for a better transparency on the consumer market in Switzerland? To answer these questions, we conducted an extensive field trial across three Swiss sites and over two years where we tested 48 spelt candidate varieties from Switzerland and Central Europe and compared their genetic, morphological, agronomic, nutritional, and baking characteristics to the two Swiss reference varieties for typical spelt (i.e. *Ostro* and *Oberkulmer Rotkorn*).

Materials and methods

Considered varieties

The 50 chosen spelt landraces and modern cultivars are described in Table S1 and represent a diversity of local (i.e. Swiss) and European varieties that were developed between 1948 and 2020, including some breeding lines that have not yet been registered on official lists. All the varieties are winter spelt, except *Flauder*, which can be grown both as winter or spring spelt.

Field trials

The field trials took place in three sites across Switzerland for two seasons, in 2020/2021 and 2021/2022. The sites were located in Changins (1260), Delley (1567), and Feldbach (8714), and are characterized by the climatic conditions described in Table S2.

Each variety was grown in a plot of 7.1 m^2 (1.5 m*4.7 m) in Changins and Delley, and 4.5 m^2 (1.5 m*3 m) in Feldbach. We replicated the experiment three times per location. At each site, we used a complete randomized block design, with plots being randomized within each block. Density of sowing was 180 spikelets/m^2. Plots were sowed mechanically each autumn. In Changins and Delley, the plots were mechanically fertilized with 100 kg N/ha (ammonium nitrate), applied in two splits (60 at heading stage—40 at flowering stage). In Feldbach, the fields were treated organically, and therefore no synthetic fertilizer was applied.

Agronomic and morphological characteristics

For each plot, we recorded the heading date as the day of the year, in which 50% of the ears of the plot had fully emerged from the flag leaf. Once the plants and ears were fully developed, plant height was measured in each plot, by taking the average height in centimeters from the ground to the top of five random ears, excluding awns.

At maturity, we harvested each plot with a combine harvester (Zürn 150, Schontal-Westernhausen, Switzerland). The harvested grains were weighed first, dehusked, sorted and cleaned with a sieve cleaner, and then weighted again. We measured specific weight and water content using a Dickey–John machine (GAC 2100). Grain yield was subsequently standardized to 15% of humidity. Protein content (%) was measured at the plot level with a near-infrared instrument (ProxiMate™, Büchi instruments). Thousand kernel weight (TKW, g), as well as kernel length and width (mm), were measured at the plot level with a Marvin seed analyzer (GTA Sensorik, Neubrandenburg, Germany).

Additional measurements in Changins: we computed harvest index for each plot by cutting 30 individual culms just before harvest. Plants were cut just above the ground, oven-dried for 3 days at 80 °C and then weighed. We then threshed, dehusked, sieved and weighed the obtained grains. The harvest index was computed by taking the ratio of grain mass over total mass. The angle of curvature of the spike was measured in 2021 to assess ear snapping: before harvest, we sampled one individual per variety and took a picture of its spike in standardized conditions. The curvature of the spike (in °) was then calculated using ImageJ.

Rheological characteristics

At all sites, Zeleny sedimentation value (mL) was assessed based on the International Association for Cereal Science and Technology standard method 116/1 (International Association for Cereal Science and Technology (ICC), 1994; Levy Häner et al. 2015).The analyses were performed by the analytical laboratory of DSP, Delley, at the variety level for each site—i.e., grains from the three replicates per site were pooled together and subsequently milled.

Additional measurements in Changins were done for each variety, based on a pooled sample of the three replicates. Extensograph properties of the obtained dough were assessed according to ICC standard method 114/1 (International Association for Cereal Science and Technology (ICC) 1994); area under curve (energy, cm2), resistance to extension at 5 cm extension (EE), and extensibility of the dough (mm) were measured. The analyses were performed by the accredited laboratory "Versuchsanstalt für Getreideverarbeitung" based in Austria (https://www.vfg.or.at/).

Nutritional characteristics

We assessed the structure of starch (amylose content) and the fatty acid composition for each variety in Changins. These analyses were done by pooling grains from the three replicates in Changins and milling them. The amylose and amylopectin contents of starch were determined enzymatically via an assay based on the precipitation of amylopectin complexes with the lectin concanavalin A, according to K-Amy 06/18 (Megazyme 2018). The fatty acid composition was analyzed by GC-FAME, via in situ transesterification, according to the method of Ampuero Kragten et al. (2014). These analyses were performed at the accredited analytical laboratory of Agroscope, Posieux.

DNA extraction

To obtain DNA from each variety, we grew 3 individuals of each variety as single plants in an outdoor experimental field in Changins in 2021. This was done by placing a dark agricultural perforated foil over the field, which contained one hole every 0.25 square meters. We then sowed three spikelets per variety in each hole. The individual plants were part of a more extended experimental setup, which contained 389 spelt and wheat accessions from various European countries for further genomic analyses (Table S3 for the list of accessions). To extract the DNA of these accessions, 50 mg of leaf material from one seedling per accession was harvested into cluster tube strips (MTS-11-8-C, Chemie Brunschwig), shock frozen, and stored at − 80 °C. DNA extraction was performed based on the protocol of Mayjonade et al. (2016) and adapted for automated purification. Bead-ground leaf tissue was lysed in 600 µl lysis buffer and incubated at 50 °C for 1 h. Then, 100 µl of 5 M potassium acetate was added, mixed, and centrifuged at 2300 g for 20 min to precipitate plant fibers and SDS-bound proteins. Subsequently, 300 µl of the supernatant was transferred to 2.2 ml deep well blocks, and 300 µl of 11% PEG binding solution containing 4 µl of washed Sera-Mag magnetic beads (GE65152105050250, Sigma) was added. All further steps for DNA binding, washing, and elution were performed using the automated KingFisher™ Apex Purification System (ThermoFisher) as follows: After a 10 min binding step, the beads were resuspended in 90 µl of 5.5% PEG binding solution before adding 300 µl of the wash solution. Subsequently, two washes with 400 µl each were performed, and the purified DNA was eluted in 100 µl of elution buffer (100 mM Tris pH 8.0, 0.1 mM EDTA).

20 µl (~ 2 µg) of the DNA were sent to TraitGenetics (SGS institute Frenius, Gatersleben DE) for SNP genotyping on the 25 K XT Infinium array for wheat.

Genotyping

The 389 accessions from the extended experimental setup were genotyped with an Illumina Infinium 25 K wheat SNP array (TraitGenetics GmbH, Gatersleben, Germany) consisting of 24,145 SNPs. 1,155 SNPs returned missing data in all accessions and were excluded from further analysis. 22 winter spelt accessions had missing data for all markers and were, subsequently, removed from further analysis. Finally, 325 and 42 winter and spring spelt accessions, respectively, were considered for further analysis. Genotyping data are available in the supplementary material (Supp.2). In order to increase diversity, sample size and discrimination power of the dataset, we further integrated genotyping data from a previous study where 502 and 293 bread wheat and spelt accessions, respectively, were genotyped with Illumina Infinium 15 K wheat SNP array (TraitGenetics GmbH, Gatersleben, Germany) consisting of 13,006 SNPs (Müller et al. 2018). Based on SNP name, 12,798 SNPs were common to both SNP arrays. 82 genotypes were genotyped in both studies, showing a high genotyping reproducibility, with less than 0.2% of wrong calls. Principal Component Analysis was performed using an R "SNPrelate" package (Zheng et al. 2012).

Statistical analyses

The main objective of this study was to see whether the genetic distinction between wheat-type and typical spelt correlated with any of the phenotypic measurements performed on the chosen 50 spelt varieties. The Principal Component Analysis (PCA) obtained from the whole genomic dataset showed that common bread wheat and spelt varieties were distinctively separated along the first axis, as previously demonstrated by Müller (2018) (Fig. S1). The values corresponding to the first axis of this PCA (PCA1) therefore allow to quantitatively discriminate varieties according to their genetic closeness to a reference wheat and/or typical spelt reference varieties. For this study, we extracted the values of the first axis of the previously-obtained PCA for the 50 spelt varieties of agronomic interest that we investigated (see Fig. 1 in the *results* section).

In a first step, we calculated correlation coefficients between the PCA1 values and each of our agronomic, morphological, or baking traits. The correlations were computed for each year separately. In a second step, we conducted a different PCA analysis on the full phenotypic dataset of Changins using the function *prcomp.* The phenotypic data included ratio protein/yield, height, harvest index (HI), heading date, thousand kernel weight, seed width/seed length, content of monounsaturated fats, content of polyunsaturated fats, ratio of oleic/palmitic fatty acids (C18.1cis9 / C16.0), content of amylose, ratio of Omega6 over Omega3 fatty acids, Zeleny, ratio Zeleny/protein, elongation after 90 min, resistance after 90 min, energy after 90 min and ratio extensibility/resistance after 90 min. We did the PCA analysis separately for each year, using averaged values over replicates. We also performed permutational multivariate analyses of variance (PERMANOVA; Anderson 2001), with Bray–Curtis dissimilarity on the previously described phenotype matrices (i.e., separated per year) using the function *adonis* from the *vegan* package with 999 permutations (Oksanen et al. 2019) and PCA1 as explanatory variable. In a third and final step, we used a partial least square regression model to assess the dependency relationship between PCA1 and our measured traits. We chose this model as partial least square regression allows us to handle multiple explanatory variables that may be correlated. This was done for the full dataset of Changins only, using the function *plsr* from the *pls* package in R (Liland et al. 2022) and including all the explanatory variables described above. We used average data from both 2021 and 2022 for each variable to train and validate the model; for this, we randomly split the data into 42 training points and 8 validating points. The optimal number of components for the model was selected based on the highest r-squared between the predictions and the validation datapoints. Furthermore, we used the VIP (Variable Importance in Projections) function from the *pls* package to rate and extract the contribution of each variable to the model accuracy. To increase the robustness, we repeated this process 100 times, each time randomly reshuffling the training and validation data. We then looked at the average VIP scores for each variable, as well as the mean r-squared of the cross-validation between predictions and validation datapoints.

Results and discussion

In the last decades, spelt (*Triticum aestivum* ssp. *spelta*) has regained interest from consumers, farmers, and bakers, as it is presented as an alternative to bread wheat (Curzon et al. 2021; Rapp et al. 2017; Tóth et al. 2022). However, the distinction between typical spelt in Switzerland and modern wheat-spelt intercrosses (i.e. derivatives of crosses between bread wheat and typical spelt) remains unclear (Koenig et al. 2015), leading to some confusion among farmers and consumers as to what they are growing and eating. Considering the growing interest in cultivating and consuming spelt, there is a need for more clarity and transparency regarding the genetic proximity of spelt cultivars to the Swiss spelt references, as well as precisions in the proper phenotypic characterization of typical spelt. To shed light on this matter, we conducted one of the largest and most complete spelt variety trials, during which we properly characterized numerous varieties of Central European spelt with different degrees of wheat crossing at both the genetic

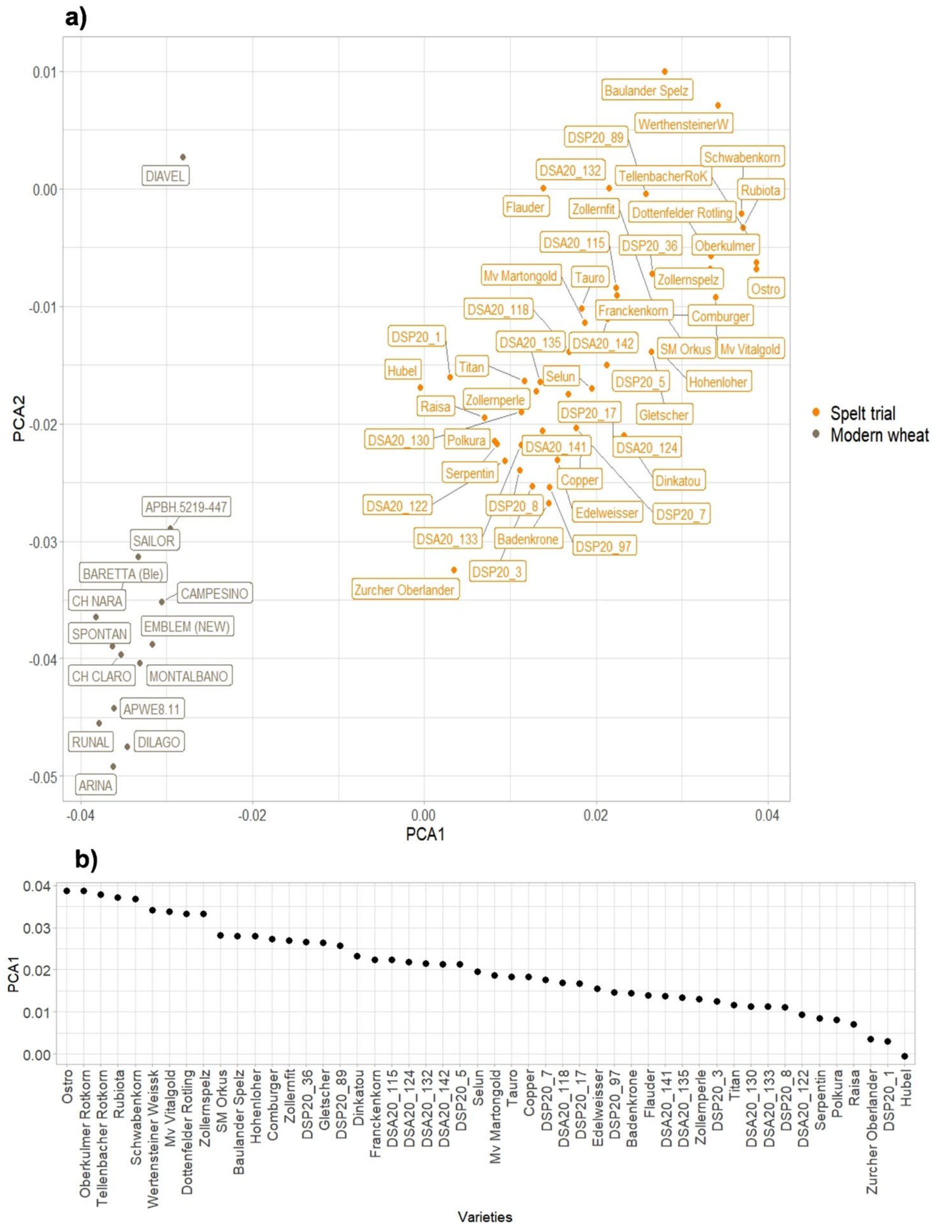

Fig. 1 **a** Subsample of the principal component analysis from Fig. S1 including the Swiss bread wheat varieties and the spelt accessions from the agronomic trials. Bread wheat (left) and spelt (right) accessions are separated. **b** Genetic proximity to reference wheat (i.e. PCA1) for the different spelt varieties. A higher PCA1 value indicates a variety closer to typical spelt (i.e. *Ostro* and *Oberkulmer Rotkorn*, with a value close to 0.04). Lower PCA1 values indicate varieties closer to modern wheat (for instance *Hubel, Zürcher Oberländer*, with values close to 0). See Fig S1 for the complete PCA graph including Swiss bread wheat

and phenotypic levels. The various analyses gave us a good picture of how to phenotypically characterize typical spelt compared to modern wheat-spelt intercrosses in the Swiss context.

Gene pools of modern Swiss bread wheat varieties and traditional Swiss spelt varieties are clearly separated

The principal component analysis revealed that common bread wheat and spelt varieties were distinctively separated along the first axis (Fig. 1 and Fig. S1), in line with previous findings from Müller (2018). We observed two distinct gene pools: modern Swiss bread wheat varieties clustered on the left side of the PCA, with *CH Nara, Arina*, and *Montalbano*, for instance. Traditional Central European spelt landraces were located on the right side, with *Ostro* and *Oberkulmer Rotkorn* on the far-right side of the PCA. The spelt group was noticeably more spread out along the first axis of the PCA, indicating that many of the spelt accessions studied are the result of a various number of crosses between traditional Swiss spelt and modern Swiss bread wheat cultivars. We will refer to these varieties originating from recent crosses between Swiss typical spelt and modern Swiss bread wheat as "modern wheat-spelt intercrosses". This is for instance the case for *Zürcher Oberländer* or *Hubel* (Fig. 1b). Importantly, this differentiation is based on genetic analyses covering the Central European genepool of spelt and wheat, and is therefore only valid in this specific context; a universal genetic distinction between *ssp. spelta* and *ssp. aestivum* cannot be made (Abrouk et al. 2021).

Correlations with variety traits

Lower yield in typical spelt is compensated by higher protein content and thousand kernel weight

PCA1 negatively correlated with yield in both 2021 and 2022 (Fig. S2, $r = -0.17$, p-value $= 0.041$ in 2021; $r = -0.27$, p-value < 0.0001 in 2022), meaning that varieties closer to typical spelt had a lower yield than varieties closer to wheat. This is consistent with many previous studies showing an average yield decrease of 30% in spelt compared to common wheat (Bonafaccia et al. 2000; Budzyński et al. 2018; Jablonskytė-Raščė et al. 2013; Longin et al. 2016). In their trial, Budzynski et al. found common wheat to have the highest yield, while the yield of spelt was 31% lower. In Jablonskyte et al., the authors witnessed a similar yield loss of 27–30% in spelt compared to common wheat. Finally, in Longin et al., spelt only reached 37% of the yield of common wheat. In our study, *Hubel* and *Zürcher Oberländer*—varieties both showing a high degree of wheat crossing—showed the highest yields across years and sites (41.9 dt/ha and 41.3, respectively). At the same time, *Oberkulmer Rotkorn* only reached 29.1 dt/ha and *Ostro* 29.6 dt/ha, resulting in a yield diminution of 29% (Table S4).

This lower yield was accompanied by higher protein content in typical spelt varieties (Gomez-Becerra et al. 2010; Koenig et al. 2015; Takač et al. 2021): there was a positive correlation between PCA1 and protein content (Fig. S3). However, this relationship was only significant in 2022 (Fig. S4, $r = 0.18$, p-value $= 0.029$). In our trials, *Oberkulmer Rotkorn* had an average protein content of 15% and *Ostro* 14.8%, while *Hubel* only reached 12.9% across sites and years (Table S4). The increase in protein content for typical spelt varieties was not a simple compensation for their lower yield (i.e. trade-off between yield and protein content (W. K. Anderson et al. 1998)): if this were the case, we would see a ratio of protein/yield constant. Here, we see an increase in the ratio protein/yield with spelt typicity in 2022 (Fig. S4, $r = 0.24$, p-value $= 0.0034$), which indicates that, when corrected for equal yield, typical spelt varieties still have higher protein content than modern wheat-spelt intercrossed varieties.

Moreover, typical spelt varieties had a higher thousand kernel weight (TKW) than wheat-type varieties in both years (Fig. S5, $r = 0.19$, p-value $= 0.018$ in 2021, $r = 0.2$, p-value $= 0.014$ in 2022), consistent with previous findings (Dziki et al. 2005; Marconi et al. 1999). TKW is an indicator of wheat milling value and can be an important criterion for millers, as samples with lower TKW values can be refused by millers (Marconi et al. 1999). Similarly, we observed differences in seed shapes in response to genetic distance: there was a strongly significant relationship between genetic distance to typical spelt and the ratio of seed width over seed length (Fig. S6, $r = -0.42$, p-value < 0.0001 in 2021, $r = -0.36$, p-value < 0.0001 in 2022), indicating that typical spelt have longer and narrower seeds than wheat-spelt intercrosses. This was also shown by Markowski (2013), who found that *Oberkulmer Rotkorn* had the highest seed length, while Curzon (2021) and Dvorak (2012) reported that spelt grains are longer and less round than wheat grains.

Typical spelt varieties grow higher, later, and invest fewer resources into grain production

Height was positively correlated with PCA1 in both years (Fig. S7, r = 0.24, *p*-value = 0.018 in 2021, r = 0.17, *p*-value = 0.043 in 2022), signifying that varieties genetically closer to typical spelt are higher than varieties genetically closer to wheat. Typical spelt varieties are indeed known to be taller than modern wheat, which is the cause of their well-known lodging problems (Longin et al. 2016). For instance, in the study of Longin (2016), spelt was about 30 cm taller than common wheat. In our field trials, the tallest varieties were *Werthensteiner Weisskorn* (140 cm), *Rubiota* (139 cm), *Schwabenkorn* (138 cm) and *Oberkulmer Rotkorn* (137 cm), which are all on the typical spelt side according to the genetic results. The shortest varieties showed a higher spread across genetic distances and for instance included *Hubel* (107 cm) and *Polkura* (105 cm)—containing a high degree of wheat crossing—but also *Zollernfit* (89 cm) and *Zollernspelz* (98 cm), which are genetically located on the typical spelt side. A reduction in height in modern wheat-spelt intercrosses makes sense, as reduction of height has been a target for breeding in the past decade in order to reduce lodging in spelt (Longin and Würschum 2014), but also to improve resource allocation to grains (Heitholt et al. 1990).

There was no significant relationship between PCA1 and the angle of curvature of the spike, assessing ear snapping (r = 0.16, *p*-value = 0.27, Fig. S8). Ear snapping is specific problem of spelt that can lead to consequent yield losses, when the ear bends downwards before harvest and risks breaking off (Longin and Würschum 2014). Previous work on spelt has associated this character with one major QTL located on chromosome 7A (Würschum et al. 2017), and emphasized the importance of the Q locus for spike morphology and fragility (Sormacheva et al. 2015). Therefore, we expected typical spelt varieties to have higher bending angles than modern varieties, as this is a trait that is not wished for in modern varieties. In our dataset, we only witnessed a trend towards higher angles with spelt typicity (Fig. S8), but this was not significant. However, this trait is known to be influenced by climatic conditions before harvest, notably by variations between hot and dry with wet weather (Longin and Würschum 2014), which could explain why we did not see any significant effect. Furthermore, we only measured this parameter in the first year and may thus lack power to reach statistical significance.

Phenology, assessed by heading date, was strongly influenced by spelt typicity: we found a significant relationship between PCA1 and heading date for both years (Fig. S9, r = 0.27, *p*-value < 0.0001 in 2021, r = 0.28, *p*-value < 0.0001 in 2022), indicating that varieties closer to typical spelt had a later heading date than varieties closer to wheat. Specifically, we observed a maximal difference of 12 days between *Flauder* and *Zürcher Oberländer* (142) and DSP20_36 and *Wertensteiner Weisskorn* (154). Typical spelt has been reported to have a later phenology caused by its sensitivity to day length and not temperatures (Curzon et al. 2021; Pandey et al. 2021; Teofilova and Kirchev 2012). This is why spelt has mainly been cultivated in northern Europe, where growing seasons are characterized by long photoperiods and cold temperatures (Longin and Würschum 2014). However, considering current climatic changes and the shortening of winter and spring seasons, typical spelt may not be well adapted to future climates; this can explain why modern spelt tend to have earlier phenology, as this might be a target for breeding (Curzon et al. 2021).

Finally, PCA1 was negatively correlated with harvest index (Fig. 2, r = −0.36, *p*-value < 0.0001 in 2021, r = −0.26, *p*-value = 0.0013 in 2022), which suggests that typical spelt had a lower rate of resource partitioning into the grain compared to modern spelt-type wheat (Dai et al. 2016). In our field trials, we observed values of HI ranging from 0.24 for *Wertensteiner Weisskorn* to 0.38 for *Badenkrone* and *Polkura. Oberkulmer Rotkorn* reached an average HI of 0.28, while Ostro had 0.3. This effect was previously reported in several agronomic studies, where lower HI values were observed for spelt compared to common wheat (Konvalina et al. 2014; Koutroubas et al. 2012; Sugár et al. 2019).

Typical spelt gives more extensible doughs than modern wheat-spelt intercrosses

Zeleny sedimentation values were negatively correlated with PCA1; however, this was only significant in 2022 (Fig. S10, r = −0.17, *p*-value = 0.033 in 2022). This indicates that typical spelt varieties had

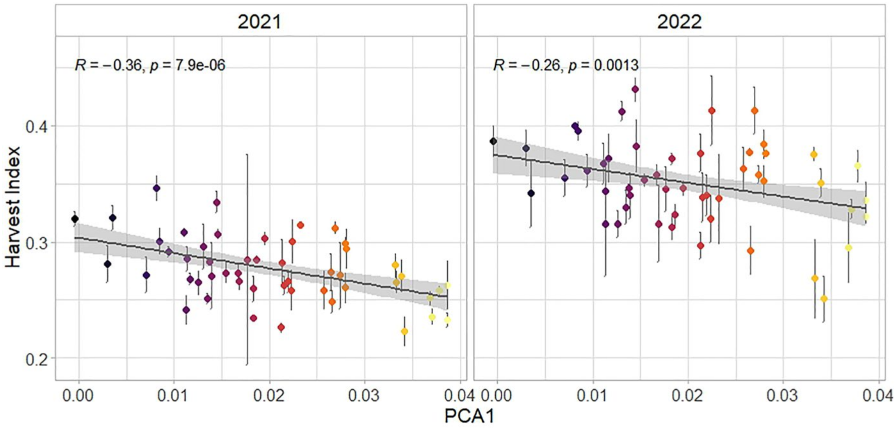

Fig. 2 Relationship between Harvest Index and genetic distance to reference wheat (PCA1, i.e. proxy for spelt typicity) for 2021 and 2022. n = 150 per year. Dots represent the mean values across replicates in Changins; lines represent the standard error. The grey lines represent linear regression fittings, with the grey area representing the 0.95 confidence interval

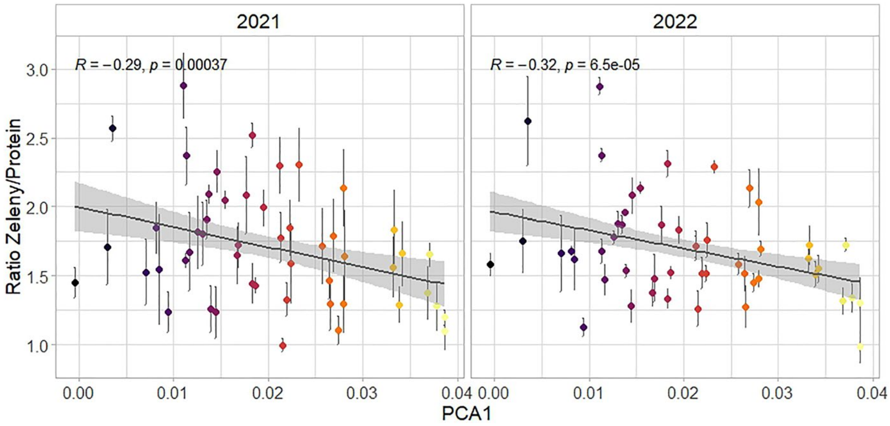

Fig. 3 Relationship between ratio Zeleny (mL)/Protein content (%) and genetic distance to reference wheat (PCA1, i.e. proxy for spelt typicity) for 2021 and 2022. n = 150 per year. Dots represent the mean values across replicates and sites; lines represent the standard error. The grey lines represent linear regression fittings, with the grey area representing the 0.95 confidence interval

a lower sedimentation value than wheat-type varieties. The Zeleny sedimentation value measures the degree of sedimentation of flour suspended in a lactic acid solution during a standard time period and is considered an indicator of baking quality (Hrušková and Faměra 2003; Levy Häner et al. 2015). More precisely, the swelling of the gluten fraction of flour in the lactic acid solution affects the rate of sedimentation; therefore, slower sedimentation and higher Zeleny values are generally associated with both a higher gluten content and a better gluten quality (Hrušková and Faměra 2003). Here, when looking at the ratio between Zeleny values and protein content, we observed a significant negative correlation in both years (Fig. 3, r = − 0.29, *p*-value < 0.0001 in 2021, r = − 0.32, *p*-value < 0.0001 in 2022), showing that for a similar content of protein, typical spelt varieties had a lower sedimentation value than wheat-type varieties. This is in line with previous studies showing that spelt had different protein fractions and protein properties compared to wheat (Frakolaki et al. 2018). More specifically, it was demonstrated that the ratio of gliadins to glutenins increased (Geisslitz et al. 2019), meaning that spelt gluten was more extensible and less elastic than gluten from common wheat, resulting in weaker doughs and low baking volume for typical spelt (Geisslitz et al. 2018; Schober et al. 2006). This was supported by our extensograph results, where both dough extensibility and ratio extensibility/resistance were correlated with spelt typicity (Fig. 4, Fig S13): notably, we found a significant positive relationship between dough extensibility after 90 min and PCA1 during both years (Fig. 4, r = 0.36, *p*-value = 0.011 in 2021, r = 0.56, *p*-value < 0.0001 in 2022). There was no significant relationship between dough energy after 90 min and PCA1 in neither of the years (Fig. S11, r = 0.037, *p*-value = 0.8 in 2021, r = 0.018, *p*-value = 0.9 in

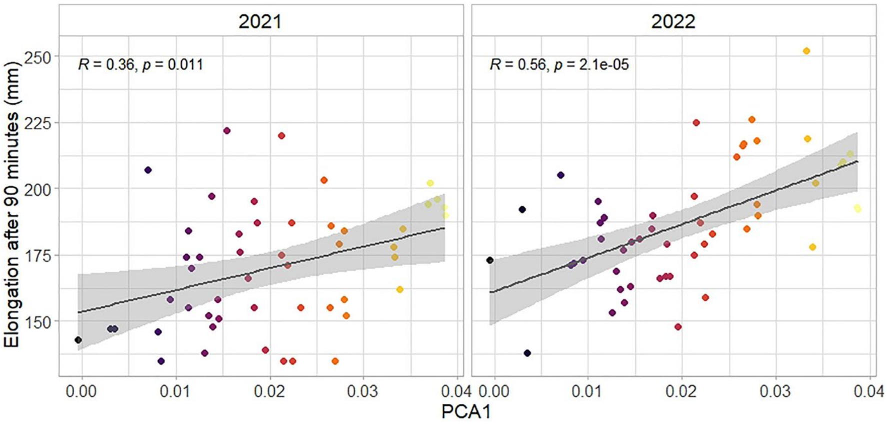

Fig. 4 Relationship between extensibility after 90 min (mm) and genetic distance to reference wheat (PCA1, i.e. proxy for spelt typicity) for 2021 and 2022 in Changins. n = 50 per year. The grey lines represent linear regression fittings, with the grey area representing the 0.95 confidence interval

2022), nor between resistance to elongation after 90 min and PCA1 (Fig. S12, $r = -0.11$, p-value = 0.44 in 2021, $r = -0.26$, p-value = 0.071 in 2022). Regarding the ratio extensibility/resistance, we found a negative relationship to PCA1 but only in 2022 (Fig. S13, $r = -0.39$, p-value = 0.0049). This suggests that typical spelt have more extensible doughs than modern wheat-spelt intercrosses; likewise, the lower ratio extensibility/resistance suggests short gluten properties and consequently, final bread products of low volume (Frakolaki et al. 2018).

Typical spelt has more unsaturated fatty acids than wheat-spelt intercrosses, while starch structure remains unchanged

We observed a significant positive relationship between the ratio of oleic/palmitic fatty acids and PCA1 in both years (Fig. S14, $r = 0.26$, p-value = 0.067 in 2021, $r = 0.44$, p-value = 0.0015 in 2022), meaning that typical spelt varieties showed a higher ratio of these two fatty acids than wheat-spelt intercrosses. The same pattern was observed between monounsaturated fatty acids content and PCA1 (Fig. S15, $r = 0.34$, p-value = 0.015 in 2021, $r = 0.37$, p-value = 0.009 in 2022), and for the ratio Omega-6/Omega-3 in 2021 (Fig. S16, $r = 0.29$, p-value = 0.044 in 2021). Higher proportion of unsaturated fatty acids as well as higher content of oleic acid in spelt have been reported previously (Relina et al. 2021; Ruibal-Mendieta et al. 2005) and constitutes a dietary and health advantage. Unsaturated fatty acids have been associated with lower risks of coronary heart disease (Golijan et al. 2019; Majewska et al. 2018), and oleic acid has been shown to produce a similar effect by lowering blood glucose and insulin levels (Ascherio 2002; Ryan et al. 2007). However, considering the very low content of fat in wheat (between 2 and 4%), these positive health effects might be of lesser importance (Narducci et al. 2019).

We did not see any link between spelt typicity and starch structure, unlike what has been hypothesized and claimed by popular press or social media (Alvarez 2021). Starch is composed of two polymers of glucose, namely amylopectin and amylose, which differ in their branching structure: amylose is essentially linear while amylopectin is branched (Magallanes-Cruz et al. 2017). The ratio of these two polymers influences starch digestibility: a higher content of amylose will increase the proportion of resistant starch, which is digested more slowly in the bowels (Ma et al. 2020). This has health benefits, as foods containing slowly digested starch can mitigate and delay the onset of diet-related diseases, such as obesity, diabetes, and colorectal cancers (Syahariza et al. 2013). In our study, starch structure was assessed through amylose content, and we had expected to find a higher amylose content in typical spelt. This was not the case in neither of the study years (Fig. S17, $r = 0.13$, p-value = 0.38 in 2021, $r = 0.13$, p-value = 0.36 in 2022), indicating that some claims about the dietary benefits of spelt in comparison to common wheat are not supported by experimental data. Further and more extensive research on starch structure, as well as dietary fibres composition, should be performed to further invalidate or not popular beliefs (Alvarez 2021).

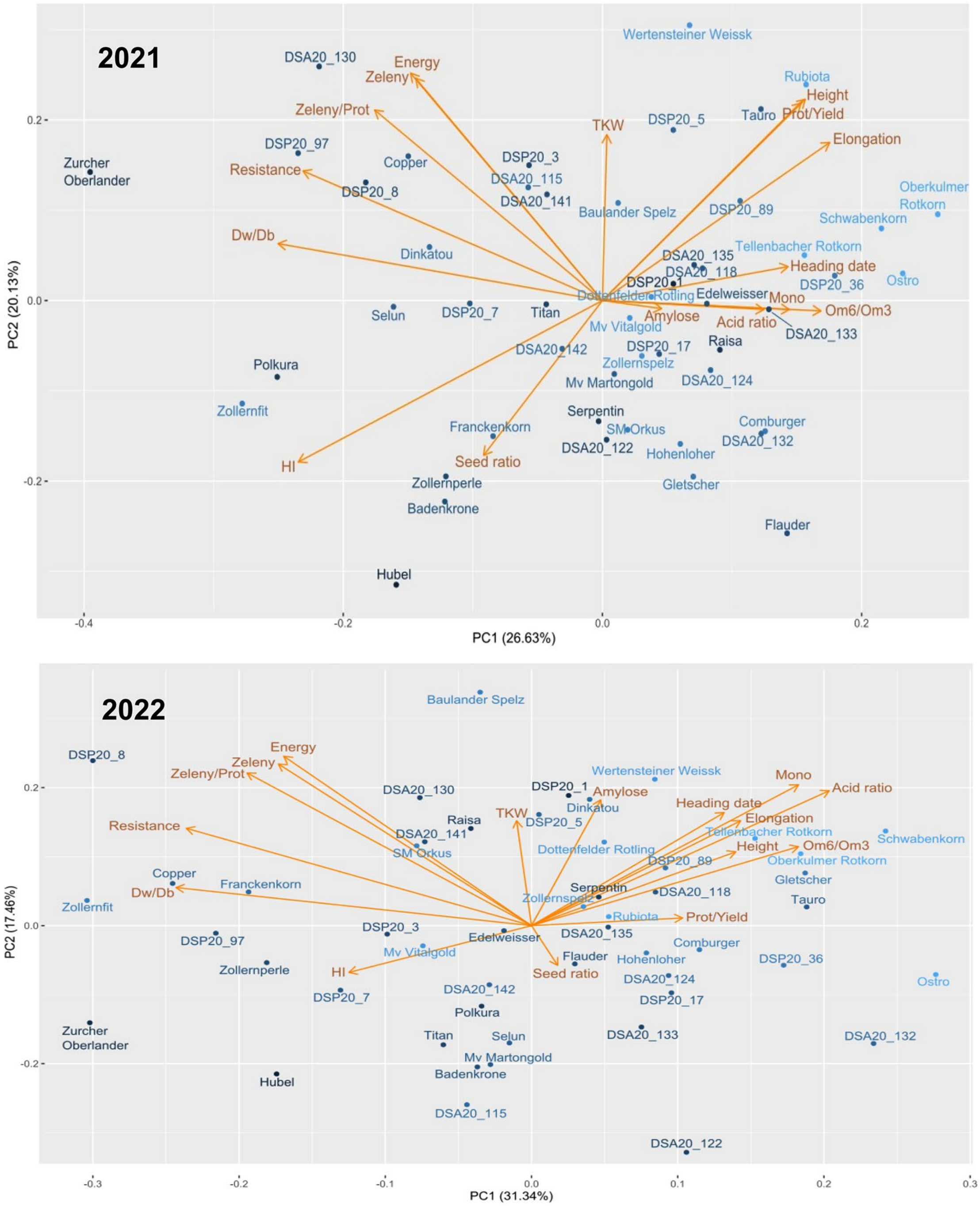

Fig. 5 Principal component analysis including all the measured phenotypic traits, in 2021 and 2022. The varieties are coloured according to their genetic distance values (PCA1). Arrows indicate the scores of each parameter, while dots indicate the position of each variety on the principal component axes

A multidimensional approach identifies key traits to distinguish typical spelt from modern wheat-spelt intercrosses in the Swiss context

We used two types of analyses to integrate all the measured parameters together; first, we did a PCA with all the phenotypic parameters. The first two

Table 1 Results of the permutational analysis of variance, showing Sum of Squares, R^2, F-value, and significance of PCA1 for the phenotype data in Changins, for 2021 and 2022

Year	Explanatory variable	Sum of Squares	R^2	F	*p*-value
2021	PCA1	0.013	0.061	3.1	**0.03***
2022	PCA1	0.023	0.094	5.0	**0.011***

Number of observations are 50 in 2021 and 50 in 2022

Bold values indicate significant responses (*p*-values < 0.05)

p-values are significant at a = 0.05

$*p<0.05$; $**p<0.01$; $***p<0.001$

principal components (PC1 and PC2) of the phenotypic PCA explain between 46% (2021) and 48% (2022) of the variation of the observed data (Fig. 5). The PCA biplot represents the projection of each sample data and their positions on the PC1 and PC2 values, as well as the projections of vectors of each parameter. Visually, we can see that most of the varieties genetically close to *Ostro* and *Oberkulmer Rotkorn* (colored in lighter blue) are gathered on the right side of the plots, while varieties genetically closer to wheat, such as *Zürcher Oberländer* and *Hubel* (in darker blue) are more on the left side. This indicates potential links between the phenotypic parameters and the degree of spelt typicity of the varieties. The permanova test confirmed the visual patterns: in both years, the trait matrix was significantly linked to the genetic value PCA1 (Table 1), even though the percentage of variation explained by the genetic distance remained low (6% in 2021 and 9% in 2022). Such low percentage can be explained by natural heterogeneity and variability inherent to field data. Furthermore, the graphs allowed us to see that heading date, elongation, height, as well as the protein/yield, oleic/palmitic, and Omega-6/Omega-3 ratios pointed towards *Ostro* and *Oberkulmer Rotkorn*. This suggests that

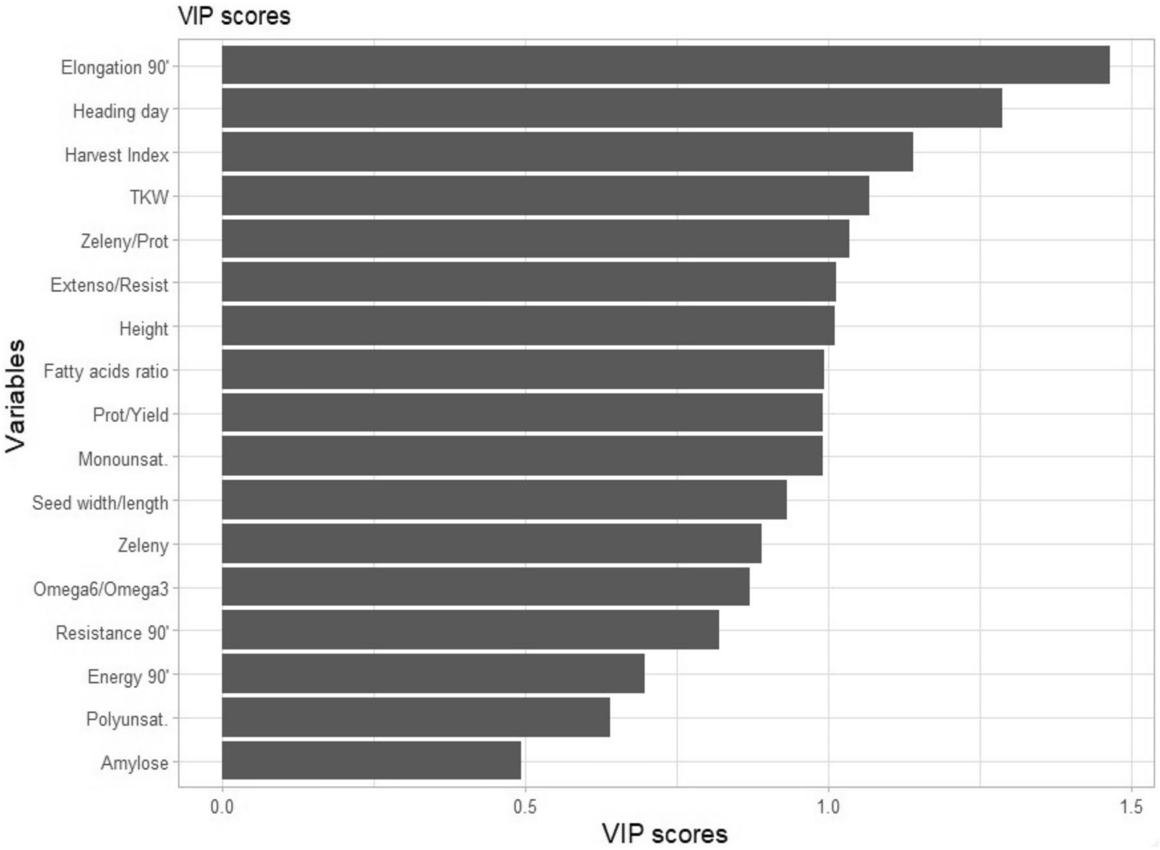

Fig. 6 VIP scores for each parameter from the partial least-squared regression model. VIP score higher than 1 indicates a significant explanatory variable

typical spelt varieties are characterized by higher values of the mentioned variables and confirms the results coming from the correlations. Similarly, we can see on the graphs that wheat-spelt intercrosses show a higher harvest index, as it points away from the typical spelt varieties.

Secondly, we ran a partial least square regression model to investigate the ability of phenotypic trait data to explain and predict the degree of spelt typicity. Results showed a mean r-squared of cross-validation between predictions and validation data of 0.53 after 100 random reshuffling. This means that the phenotypic data could properly explain 53% of the variability in genetic distance, which is very acceptable when considering the natural heterogeneity of field data. To our knowledge, no other studies have used a similar approach, but when looking at agronomy trials using plsr models, we found similar ranges of variation explained (El-Hendawy et al. 2019; Hu et al. 2018; Mkhabela et al. 2018).

Furthermore, the PLSR model allowed to score and rank the explanatory variables by order of importance (Fig. 6). Variables with a VIP (Variable Importance in the Projection) score greater than 1 are indeed considered significant (Chong and Jun 2005; Wang et al. 2015). We found the highest average VIP scores obtained for extensibility after 90 min, heading day, harvest index, thousand kernel weight, and ratio of Zeleny/protein, which is in line with our previous analyses and suggests that spelt typicity is best characterized by a combination of baking, agronomic, and morphological traits. Regarding nutritional composition, typical spelt varieties demonstrated a higher oleic/palmitic acid ratio, a higher content of monounsaturated fatty acids, as well as a higher ratio of Omega-6/Omega-3. Despite significant correlations between these parameters and the genetic distance to wheat, the nutritional variables did not achieve high importance scores in the explanatory model, which indicates that nutritional composition alone does not properly characterize typical spelt.

Conclusion

This study allowed us to better characterize the phenotypic markers of typical spelt in the Swiss context. Our results show that in Switzerland, typical spelt is characterized by highly extensible dough, later phenology, low harvest index, high thousand kernel weights, and lower Zeleny/protein ratio. Our set of phenotypic parameters allowed us to correctly explain more than 50% of the variability in spelt typicity. By linking phenotypic characteristics, which are easily measured in field trials, to the underlying genetic information of each variety, we believe that these results will help to better categorise spelt varieties in Switzerland and pave the way for more clarity and transparency on the Swiss spelt market for farmers, bakers, and consumers.

Acknowledgements We thank Yann Imhoff, Noémie Schaad, Julie Roux, Franca Dell'Avo and Flavio Foiada for their assistance with field experiments, and Monika Baumann for support with the provisions of accessions. This project was jointly funded by the Swiss Federal Office for Agriculture (BLW) and IG Dinkel.

Author contributions LLH, KHC, and TK designed the experiment. LLH and KHC provided seeds of accessions. LS and SS organised and performed the field experiments and collected the phenotypic data. BK and GH handled the DNA extraction and SNP genotyping. SKA performed and interpreted the nutritional analyses. LS and JSM analysed the data, with support from BK and SGK. The manuscript was written by LS. All authors read and approved the final manuscript.

Funding This project was jointly funded by the Swiss Federal Office for Agriculture (BLW) and IG Dinkel.

Data availability The phenotypic data is available on Zenodo: https://zenodo.org/doi/10.5281/zenodo.13318801. The sequencing data are already available in Supplement files.

Declarations

Conflict of interests The authors declare that they have no conflict of interest.

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