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Aroma and quality of breads baked from old and modern wheat varieties and their prediction from genomic and flour-based metabolite profiles

Friedrich Longin[a], Heiner Beck[b], Hermann Gütler[c], Wendelin Heilig[d], Michael Kleinert[e], Matthias Rapp[a], Norman Philipp[f], Alexander Erban[g], Dominik Brilhaus[h,i,j], Tabea Mettler-Altmann[h,i,j], Benjamin Stich[j,k,*]

[a] State Plant Breeding Institute, Univ. of Hohenheim, 70599 Stuttgart, Germany
[b] BeckaBeck, 72587 Römerstein, Germany
[c] Stelzenmühle, 88410 Bad Wurzach, Germany
[d] Kreislandwirtschaftsamt, 72525 Münsingen, Germany
[e] Institute of Food and Beverage Innovation, Zurich University of Applied Sciences, 8820 Wädenswil, Switzerland
[f] Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research(IPK), 06466 Gatersleben, Germany
[g] Department of Molecular Physiology, Max-Planck-Institute of Molecular Plant Physiology, 14476 Golm, Germany
[h] Institute of Plant Biochemistry, Heinrich Heine University, 40225 Düsseldorf, Germany
[i] Plant Metabolism and Metabolomics Laboratory, Heinrich Heine University, 40225 Düsseldorf, Germany
[j] Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University, 40225 Düsseldorf, Germany
[k] Institute of Quantitative Genetics and Genomics of Plants, Heinrich Heine University, 40225 Düsseldorf, Germany

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ABSTRACT

Bread aroma is the principal characteristic perceived by the consumer yet it is mostly disregarded in the product chain. The main aim of this study was to evaluate the potential to include bread aroma as a new target criterion into the wheat product chain. The objectives of our study were to (i) quantify the influence of genetic versus environmental factors on the bread aroma and quality characteristics, (ii) evaluate whether bread baked from modern wheat varieties differ in terms of aroma from those baked from old varieties, and (iii) compare genomic and metabolomic approaches for their efficiency to predict bread aroma and quality characteristics in a wheat breeding program. Agronomic characters as well as bread aroma and quality traits were assessed for 18 old and 22 modern winter wheat varieties evaluated at up to three locations in Germany. Metabolite profiles of all 120 flour samples were collected using a 7200 GC-QTOF. Considerable differences in the adjusted entry means for all examined bread aroma and quality characters were observed. For aroma, which was rated on a scale from 1 to 9, the adjusted entry means varied for the 40 wheat varieties between 3 and 8. In contrast, the aroma of bread prepared from old and modern wheat varieties did not differ significantly ($P < 0.05$). Bread aroma was not significantly ($P < 0.05$) correlated with grain yield, which suggested that it is possible to select for the former character in wheat breeding programs without reducing the gain of selection for the latter. Finally, we have shown that bread aroma can be better predicted using a combination of metabolite and SNP genotyping profiles instead of the SNP genotyping profile only. In conclusion, we have illustrated possibilities to increase the quality of wheat for consumers in the product chain.

1. Introduction

Almost 700 million tons of wheat (*Triticum aestivum* ssp. *aestivum*) are produced annually around the globe (Shewry & Hey, 2015), relying on thousands of different varieties bred by hundreds of different plant breeders in public and private institutes. Wheat is the third most important staple crop on earth, after maize and rice, providing about 20% of the daily protein and calorie requirement (www.wheatinitiative.org). The wheat required for human nutrition is mostly transformed into bread before consumption, leading to the annual consumption of >9 billion kg of bread in the world (Pico, Bernal, & Gómez, 2015). Bread aroma is one of the first characteristics perceived by consumers (Pico et al., 2015). However, the potential of wheat varieties to contribute to aromatic breads is mostly ignored during the breeding, growing, and

* Corresponding author at: Institute of Quantitative Genetics and Genomics of Plants, Heinrich Heine University, 40225 Düsseldorf, Germany.
E-mail address: benjamin.stich@hhu.de (B. Stich).

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trading process. Instead, with only slight differences among countries, the main evaluation criteria are agronomic performance, resistance against diseases, and bread-making quality (cf. Laidig et al., 2017, for Germany). In the international trading of wheat, the evaluation of wheat batches is further reduced to the protein and moisture content as well as mycotoxin concentration.

Beside the intensity of milling the wheat grain, the type of dough preparation, the baking process, and the ingredients used during the dough preparation strongly influence the aroma of breads (Pico et al., 2015; Ficco et al., 2017). Starr et al. have shown that not only the aroma of the wheat grain (2013) but also the aroma of porridge and bread crumb (2015) was influenced by the chosen wheat variety. The authors concluded that the aroma of bread could become an important future characteristic for the baking industry and, thus, wheat breeding. For spelt wheat, Rapp et al. (2017) assessed the bread-making quality as well as the flavor and odor of breads prepared from 30 varieties grown at one location. The authors showed that a significant genetic variability for flavor and odor existed among the spelt varieties and that these characteristics could be combined with high agronomic performance and baking quality. However, a study investigating a higher number of wheat varieties grown at different locations with respect to the agronomic and baking properties as well as flavor and odor of their breads is still lacking.

Breeding for bread aroma and quality characteristics is hampered by the high effort necessary to assess these traits in baking trials. This is an ideal situation for the use of genomic prediction, where a model is trained based on the phenotypic information available for a subset of the genotypes and the performance of the remaining genotypes is predicted from molecular marker information (Meuwissen, Hayes, & Goddard, 2001). However, to the best of our knowledge, such approaches have not been evaluated in the context of aroma traits of wheat.

Of all the molecular entities (e.g., genes, transcripts, proteins, metabolites), metabolites have the closest relationship to expressed phenotypes as they are the end-points of upstream biochemical processing (Rattray et al., 2018). Therefore, they have recently received attention in the context of studying complex characteristics such as the bread aroma. Yan et al. (2019) evaluated the key aroma compounds in Chinese steamed bread by a metabolomics approach and identified 13 compounds that discriminate breads prepared with type I sourdough vs. baker's yeast. A study based on four durum varieties grown at multiple field locations has illustrated the high environmental impact on the expression of metabolites in the flour (Beleggia et al., 2013). However, to the best of our knowledge, no earlier study has evaluated the prediction ability of metabolome profiles generated from flour for bread aroma and quality characteristics which could considerably facilitate their alteration by breeding.

Recently, a distorted picture of wheat-based food has been promulgated in the mass media, leading to unsubstantiated concerns about its safety and health implications (Brouns, Gilissen, Shewry, & van Straaten, 2015). In addition, adoption of modern varieties has been indicated as the main reason for the loss of the sensory properties of pasta and breads (Rapp et al., 2017; Ficco et al., 2017). However, a powerful evaluation of this hypothesis based on several modern and old wheat varieties grown in the same field but across multiple locations is lacking.

The main aim of this study was to evaluate the potential to include bread aroma as a new target criterion into the wheat product chain. The objectives of our study were to (i) quantify the influence of genetic versus environmental factors on the bread aroma and quality characteristics, (ii) evaluate whether bread baked from modern wheat varieties differ in terms of aroma from those baked from old varieties, and (iii) compare genomic and metabolomic approaches for their efficiency to predict bread aroma and quality characteristics in a wheat breeding program.

2. Materials and methods

2.1. Plant material, bread baking, and its evaluation

A total of 18 old (year of release 1962–1999) and 22 modern (2005–2014) winter wheat varieties were used for this study (Suppl. Fig. 2). All varieties, which will be designated in the following as genotypes, have been cultivated in the season 2015/16 in 5 m^2 plots in Germany at $o = 3$ locations. Two locations were located in Baden-Wuerttemberg, namely Hohenheim (HOH) and Ihinger Hof (IHO), as well as at Gatersleben (GAL) in Saxony-Anhalt. The experimental design was an incomplete block design with one replication per location. At each location, days to heading (HD), plant height (PH), and yield (YD) were assessed.

The grains harvested from all 40 wheat varieties at HOH and GAL ($o = 2$) were characterized for the raw protein content (RP) using Near-infrared spectroscopy (NIRS, ICC-Standard-Method 159) (Hourston, Ignatz, Reith, Leubner-Metzger, & Steinbrecher, 2017), the volume of sedimentation according to Zeleny (SD, ICC-Standard-Method 116/1), and the falling number according to Hagberg (FN, ICC-Standard-Method 107/1). Furthermore, the wheat whole grain flour created by milling the grains from the 80 samples was used for a bread baking trial. The baking trial was performed with 1800 g of flour, 1.26 l of water, 27 g of yeast, and 36 g of salt. The ingredients were combined, mixed for four minutes on a low level and then kneaded on a high level for 30 s. After 30 min of dough rest, the dough was lifted and folded. This procedure was repeated twice and afterwards the dough was left to rest again for 60 min. Afterwards,an experienced baker evaluated the dough humiditiy (DH) and its quality (DQ) on a scale from 1 (low humidity/quality) to 9 (high humidity/quality) by stretching it manually. The dough of each genotype of each of the two locations GAL and HOH was then formed to three breads and baked as freely set and moistened bread at a temperature of 250°C for 50 min.

The breads were evaluated one day after made as then a better differentiation could be realized. The evaluation was performed according to the rules of the German Agricultural Society (DLG e.V.; DIN 10969:2001-05, DIN 10964:2014-11, and DIN 10975:2005-04), which is the standard method for testing bread-making quality in Germany and performed by almost all bakeries regularly in the context of quality management. The evaluation was performed as a consensus profiling using a panel of six trained people which gave a jointly decided evaluation of the bread aroma which comprised the following aspects: The odor (OD) was tested by closely smelling the crumb, the flavor (FL) was assessed by tasting it. The intensity of the aroma was rated on a scale from 1 = no aroma to 9 = very intense aroma, and the negative aroma compounds were noted as well. However, in our study no negative aromas were smelled/tasted and, thus, the scores for OD and FL represent the intensities of the pleasant aroma components.

Furthermore, the color of the crust (CC), the hardness of the crust (CH), the pore structure (PS), the crumb moisture (CM), the crumb elasticity (CE), and the volume of the bread (BV) were rated on a scale from 1 to 9 (Table 1). Finally, the baking quality was determined as the height/width ratio of the breads at their middle slice. To facilitate the understanding, we designated in the following all assessments of physical flour, dough, and bread characteristics as quality characteristics where odor and flavor were designated as aroma characteristics.

All wheat genotypes have been genotyped by TraitGenetics (Gatersleben, Germany) using a custom 15K SNP array that comprises a subset of the SNPs of the 90K SNPs array (Wang et al., 2014). After performing quality checks and mean imputation of the remaining data points, a set of 10,801 polymorphic SNPs was obtained. This set of predictors, designated in the following as S, was used for all further analyses.

Table 1
Summary statistics, genetic (σ_g^2) and error (σ_e^2) variance components, as well as heritability (h^2) estimates for the traits examined in this study.

Character	Abbreviation	Scale	Adjusted entry mean			Variance components		Heritability
			Min	Mean	Max	σ_g^2	σ_e^2	h2
Falling number	FN	s	66.0	329	425	4433***	3898	0.69
Raw protein content	RP	%	10.3	11.6	12.9	0.238***	0.274	0.63
Volume of sedimentation	SD	ml	12.0	29.3	47.8	59.6***	15.8	0.88
Dough quality	DQ	Low elasticity = 1; high elasticity = 9	2.0	6.2	9.5	2.62***	2.75	0.66
Dough humidity	DH	Humid = 1; dry = 9	1.5	3.9	7.5	3.01***	1.70	0.78
Crust color	CC	Light = 1; dark = 9	2.0	4.1	8.5	0.960*	1.51	0.56
Crust hardness	CH	Soft = 1; crispy = 9	5.0	7.2	8.0	0.179	0.652	0.35
Pore structure	PS	Porous = 1; dense = 9	3.1	6.1	8.5	1.26**	1.35	0.64
Crumb elasticity	CE	Soft = 1; hard = 9	1.0	6.3	8.5	1.22*	1.97	0.55
Crumb moisture	CM	Dry = 1; moistly = 9	4.5	6.1	9.0	0.459*	0.753	0.54
Bread volume	BV	Small = 1; big = 9	1.5	6.4	9.0	1.21*	1.88	0.56
Height/width ratio	HW	0.29	0.53	0.69	0.0026*	0.0045	0.53	
Odor	OD	Flavorless = 1; very aromatic = 9	3.5	6.4	8.5	0.532	1.63	0.39
Flavor	FL	Unsavory = 1; flavorful = 9	3.0	5.5	8.0	0.829	2.31	0.41
Days to heading	HD	Days after January 1st	151	156	160	5.05***	0.963	0.91
Plant height	PH	cm	65.7	80.8	94.6	44.4***	17.0	0.89
Yield	YD	dt/ha	70.3	82.8	97.6	51.0***	46.1	0.77

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.

2.2. Metabolite analyses

The metabolite analyses of our study were based on the wheat flour samples collected for the 40 wheat varieties from all three locations. Each of the 120 samples was analyzed one time via gas chromatography-mass spectrometry (GC-MS) using an adapted protocol from Lisec, Schauer, Kopka, Willmitzer, and Fernie (2006). Metabolites were extracted from 45 to 55 mg dry flour samples with 750 μl of a 1:2.5:1 H_2O:methanol:chloroform (v:v:v) mixture pre-cooled to −20 °C, then mixed on a rotator for 10 min and centrifuged at 20,000*g* for 2 min (both at 4 °C). A total of 50 μl of the supernatant were dried completely in a vacuum concentrator and derivatized in two steps via an MPS-Dual-head autosampler (Gerstel): (1) with 10 μl methoxyamine hydrochloride (Acros organics; freshly prepared at 20 mg/ml in pure pyridine (Sigma-Aldrich)) and shaking at 37 °C for 90 min, (2) adding 90 μl N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA; Macherey-Nagel) and shaking at 37 °C for 30 min. After incubation for 2 h at room temperature, 1 μl of derivatized compounds was injected at a flow of 1 ml/min with an automatic liner exchange system in conjunction with a cold injection system (Gerstel) in splitless mode (ramping from 50 °C to 250 °C at 12 °C/s) into the GC. Chromatography was performed using a 7890B GC system (Agilent Technologies) with a 30 m long, 0.25 mm internal diameter, HP-5MS column with 5% phenyl methyl siloxane film (Agilent 19091S-433). The oven temperature was held constant at 70 °C for 2 min and then ramped at 12.5 °C/min to 320 °C at which it was held constant for 5 min; resulting in a total run time of 27 min.

Metabolites were ionized with an electron impact source at 70 V and 200 °C source temperature and recorded in a mass range of m/z 60 to m/z 800 at 20 scans per second with a 7200 GC-QTOF (Agilent Technologies). Raw data files exported from MassHunter Qualitative (v b07, Agilent Technologies) in the mzData format (*mzdata.xml) were converted to the NetCDF format (*.cdf) and baseline-corrected via MetAlign (v 041012, Lommen, 2009) using default parameters. Baseline-correction was visually inspected using OpenChrom (v 1.3.0, Wenig & Odermatt, 2010). Quantitative analysis of GC-MS-based metabolite profiling experiments was then performed using TagFinder (v 4.1, Luedemann, Strassburg, Erban, & Kopka, 2008). After evaluating the uniqueness and linearity of each fragment, the aggregated fragment intensity was calculated as the average of the maximum scaled fragment intensity. For relative quantification, aggregated fragment intensities of the compounds were normalized to those of the internal standard ribitol (Sigma-Aldrich) which was added to the extraction buffer. Mass spectral annotation was manually supervised using the Golm Metabolome Database mass-spectral library (http://gmd.mpimp-golm.mpg.de/download/) after conversion of absolute time in retention indices (Strehmel, Hummel, Erban, Strassburg, & Kopka, 2008). The raw data, details of the quantification and annotation steps, and the processed metabolite profiles are available (https://doi.org/10.17632/dyfsdcxkw3.2).

The analytes that corresponded to contaminations were removed. Furthermore, if several analytes from the same metabolite were identified, the one with the higher heritability, for which the calculation is described below, was retained. The adjusted entry means of the remaining 104 metabolites across GAL and HOH for all the 40 wheat genotypes, for which the calculation is described below, was designated as M. In addition, we also used the metabolite profiles observed for the individual locations and these were designated as M_{HOH}, M_{GAL}, and M_{IHO}.

2.3. Statistical analyses

2.3.1. Phenotypic characters and metabolites

Upon the removal of outliers, each of the assessed phenotypic traits as well as the metabolites were analyzed across the locations using the following mixed model:

$$y_{ijk} = \mu + b_k{:}\, l_j + l_j + g_i + e_{ijk}, \quad (1)$$

where y_{ijk} was the observed phenotypic trait or metabolite for the ith wheat genotype in the jth location that was growing in the kth block, μ the general mean, $b_k{:}\, l_j$ the effect of the kth block nested within the jth location, l_j the effect of the jth location, g_i the effect of the ith genotype, and e_{ijk} the residual error. To estimate adjusted entry means for all genotypes, g_i and l_j were considered as fixed and all other effects as random. Furthermore, all effects, except l_j, were considered as random to estimate the genotypic variance (σ_g^2) and the error variance (σ_e^2). Heritability on an entry mean basis was calculated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/o)$.

2.3.2. Structuration and correlations

Principal component analyses were performed on the basis of (i) bread aroma and quality characteristics collected for each genotype at each of the two locations HOH and GAL, (ii) M_{HOH}, M_{GAL}, and M_{IHO}, as well as (iii) the SNP genotyping profile S. For each of the three analyses, the individual variables were scaled and centered. Correlations between different characteristics were assessed as Pearson correlation coefficient.

2.3.3. ⋆omic prediction

All prediction scenarios examined in this study relied on genomic best linear unbiased prediction (GBLUP, (Meuwissen et al., 2001)). In our study, only additive effects were modeled and the residuals $\sim\mathcal{N}(0, \sigma_e^2)$.

In order to assess the effect of predictors with a low heritability, the adjusted entry means of the wheat genotypes were predicted in a first step using M, where only those metabolites with a heritability on an entry mean basis across UHOH and GAL >0, > 0.05, > 0.10, > 0.25 (M, $M_{0.05}$, $M_{0.10}$, $M_{0.25}$) were considered. **W** is a matrix of feature measurements for the respective predictor. The dimension of **W** is determined by the number of wheat genotypes and the number of features in the corresponding predictor (m_M = 104, $m_{M_{0.05}}$ = 93, $m_{M_{0.10}}$ = 90, $m_{M_{0.25}}$ = 84). The columns in **W** were centered and standardized to unit variance. For each predictor, an additive relationship matrix was defined as

$$\mathbf{G} = 1/m * \mathbf{W}\mathbf{W}^T, \tag{2}$$

where $\mathbf{W}^T$ denotes the transpose of **W** (VanRaden et al., 2009). We calculated the prediction ability $r_{(\hat{g},y)}$ as Pearson correlation between the phenotypes and the genotypic values estimated on the basis of genomic and/or metabolomic information.

In the second step, the adjusted entry means of the wheat genotypes were predicted using combinations of metabolite profiles M and SNP genotyping profiles S, where m_S = 10,801. In accordance with Schrag et al. (2018), additive relationship matrices **G** of both predictors were established by weighting and adding up the individual matrices. The matrices thus created will be designated in the following as joined weighted relationship matrices. The relative weight of M for the calculation of the joined weighted relationship matrix was designated in our study as w, where the weight of S was 1-w. A grid search, varying w from 0 to 1 in increments of 0.05, resulted in 21 different joined weighted relationship matrices. In order to answer the question, how predictive metabolite profiles collected from individual locations were, we also used M_{HOH}, M_{GAL}, and M_{IHO} for predictions.

The standard scheme for validation of genomic prediction was a fivefold cross-validation that was replicated 200 times. The median of the prediction ability across the 1000 cross-validation runs was calculated. For all examined scenarios, the same 1000 cross-validation assignments were used.

In addition to the above described prediction of adjusted entry means of bread aroma and quality characteristics across multiple locations, we were interested in finding out whether the prediction allows the precise selection of wheat genotypes in different environments. Therefore, we assessed the prediction of location specific characteristics of the 40 wheat genotypes using the following mixed model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z_G}\mathbf{u} + \mathbf{Z_{GL}}\mathbf{v} + \mathbf{Z_S}\mathbf{t} + \mathbf{e}, \tag{3}$$

where **y** is the vector of phenotypic observations, **X** is the incidence matrix for the fixed effects, $\mathbf{Z_G}$ is the incidence matrix relating each genotype to its random additive genetic effect, and $\mathbf{Z_{GL}}$ is the incidence matrix relating each genotype to different locations. $\mathbf{Z_S}$ is the incidence matrix relating each genotype⁎location combination, which we designated as the sample in the following, to its phenotypic observation. $\boldsymbol{\beta}$ is a vector of fixed effects (in this case including general mean and location effects), **u** a vector of random additive genetic effects $\sim\mathcal{N}(0, \sigma_g^2\mathbf{G})$, **v** the vector of random genotype⁎location interaction effects $\sim\mathcal{N}(0, \sigma_v^2\,\mathbf{I}\otimes\mathbf{G})$, where **I** is a 2⁎2 identity matrix, and **t** is the vector of random sample effects $\sim\mathcal{N}(0, \sigma_t^2\mathbf{H})$. In addition, e is a vector of residuals with each element $\sim\mathcal{N}(0, \sigma_e^2)$.

We examined four different versions of the above described model. A1 was the baseline approach which corresponds to current standards in predicting genotype⁎location interaction (Jarquin et al., 2017). In this approach, genomic covariance between genotypes was modeled for the terms genotypes but also for genotype⁎location interactions. In detail, for A1, **G** was calculated from SNP genotyping profile S only and **H** was an 80⁎80 identity matrix. A2 was a modification of A1 in the sense that **G** was calculated as the optimal combination of S and the adjusted entry means of metabolites M as described above. For A3 and A4, the location specific metabolome profiles M_{HOH} and M_{GAL} were used to model the covariance between samples across the two locations. For A3, **G** was calculated from S only and **H** was an additive relationship matrix calculated as described above from a matrix of metabolomic features **V** observed in each of the 80 samples, where

$$\mathbf{V} = \begin{pmatrix} \mathrm{M}_{HOH} \\ \mathrm{M}_{GAL} \end{pmatrix}. \tag{4}$$

For A4, **G** was calculated as the optimal combination of S and M as described above and **H** was calculated from **V** as described before.

For the prediction of location specific characteristics, the breeding values were estimated as $\mathbf{u} + \mathbf{v}_j$, where $\mathbf{v}_j$ is the location specific interaction effect. We calculated then the prediction ability as the Pearson correlation between the phenotypes and the estimated genotypic values for each of the two locations and averaged the correlation across the locations. A cross-validation procedure was used as described above.

If not described differently, all analyses were performed with statistical software R (R Development Core Team, 2016).

3. Results

We observed large differences in the adjusted entry means for the different characteristics leading to highly significant genetic variances for almost all traits (Table 1). Furthermore, medium (h^2 = 0.35 for crust hardness) to high (h^2 = 0.88 for sedimentation volume) heritabilities were determined for the quality characters. The heritabilities observed for the three agronomic traits were with values of 0.77–0.91 even higher. This underpins the reliability of the field and laboratory analyses and makes our data set an ideal backbone for a deeper investigation of the metabolome and genome of the respective wheat varieties.

In the principal component analysis of the 40 wheat genotypes based on SNP genotyping profiles, no obvious clustering of the genotypes was observed with respect to the first two principal components (Suppl. Fig. 2). In the principal component analysis of the 80 bread samples based on the bread aroma and quality characteristics, the first two principal components explained 28% and 17% of the variance (Fig. 1). The samples of the two investigated locations HOH and GAL clearly separated from each other with respect to the second principal component. This differentiation was mainly caused by differences in protein content, crust hardness, crust color, sedimentation volume, and bread volume.

Phenotypic correlation coefficients varied largely between the different bread aroma and quality characteristics as well as agronomic traits (Suppl. Fig. 3). For instance, the highly significant ($P < 0.05$) negative correlation between yield and protein content was confirmed in this data set. In contrast, the sedimentation volume and the dough quality correlated slightly positive with grain yield. This is of high interest as both characters positively correlated ($P < 0.05$) with the final baking quality as measured by the bread volume or the height/width ratio, while the protein content did not. This finding suggests that the combination of high grain yield with high baking quality is possible and can be evaluated in breeding programs via the proxy sedimentation volume and by the baker via an easy dough quality test. Odor and flavor of bread correlated positively with each other (Suppl. Fig. 3). However, both were not significantly ($P < 0.05$) correlated with grain yield (Fig. 2). By contrast, odor was significantly ($P < 0.05$) correlated with bread volume and height/width ratio. A significant ($P < 0.05$) difference between the average yield and the height/width ratio of old and modern wheat varieties was observed (Fig. 3) which illustrates the selection gain realized over the last decades. However such a difference was not observed for odor and flavor.

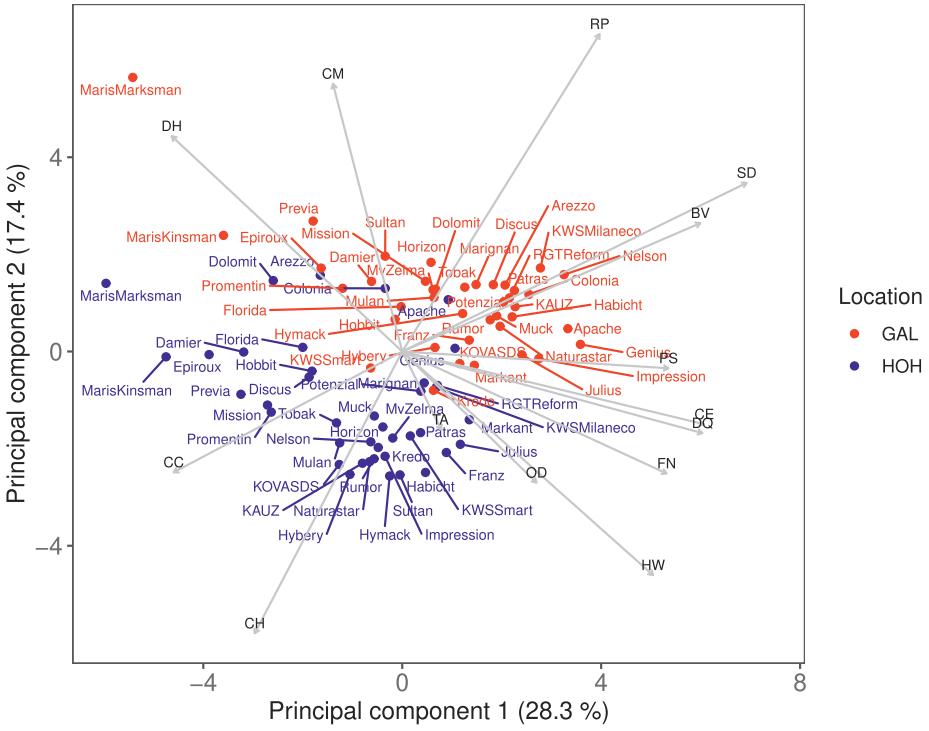

Fig. 1. Principal component (PC) analysis biplot of 40 wheat genotypes for their bread aroma and quality characteristics (for abbreviations, see Table 1) across two locations. The arrows represent the bread aroma and quality characteristics. The numbers in parentheses refer to the proportion of variance explained by the PC.

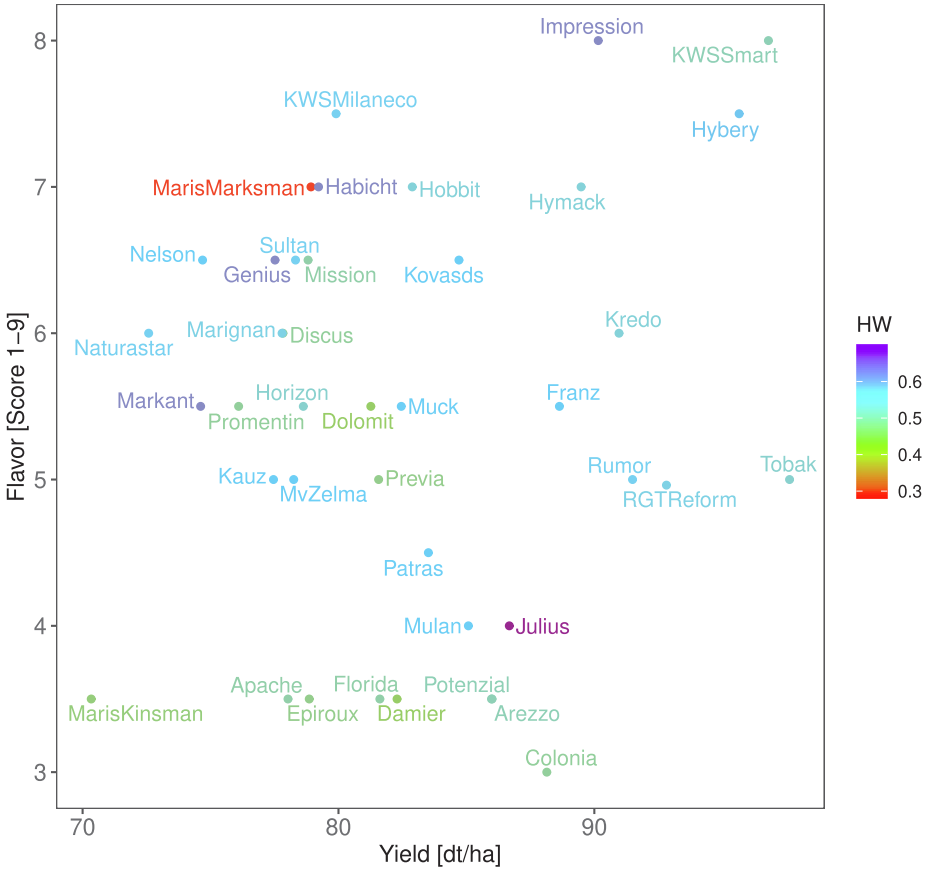

Fig. 2. Correlation biplot of the adjusted entry means of the 40 wheat genotypes for flavor versus yield. The colors indicate the adjusted entry mean of the height/width ratio (HW) of the bread.

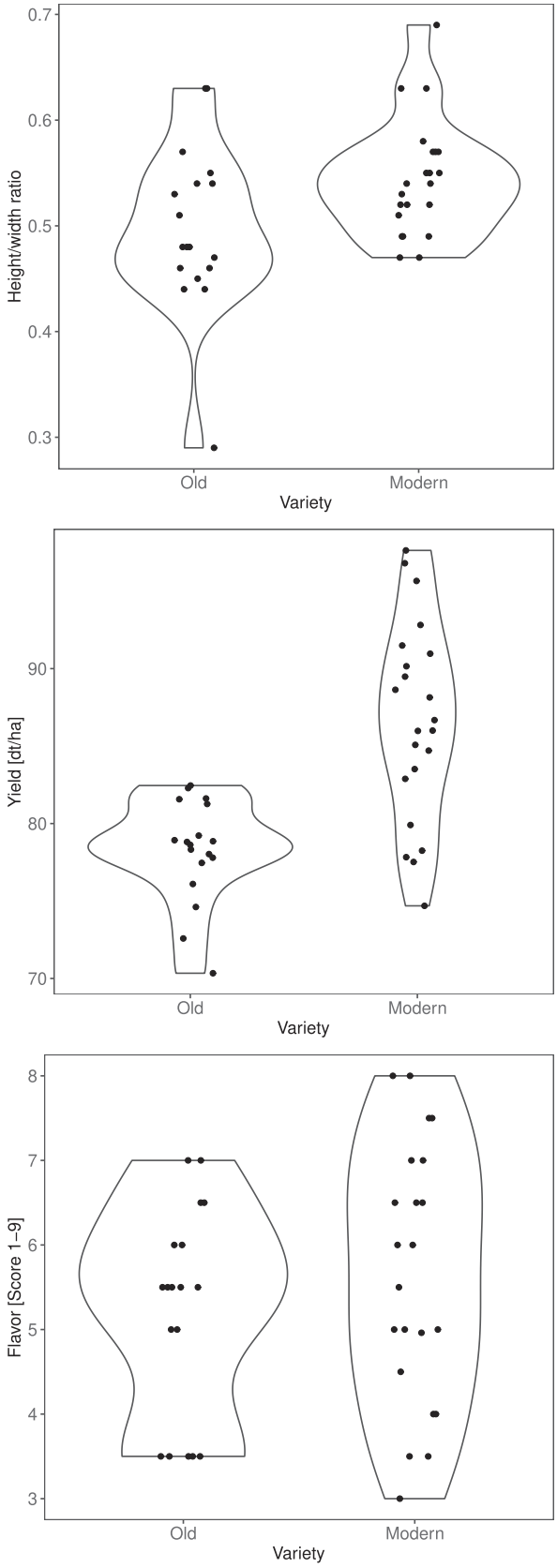

Fig. 3. Violin plots of the adjusted entry means of the 40 wheat genotypes grouped based on the year of release in old and modern varieties for height/width ratio, yield, and flavor.

A total of 149 analytes were annotated (Suppl. Fig. 4). After filtering, 104 metabolites remained for which the relative abundances were used for our analyses. The set of metabolites was comprised of 20 organic acids, 17 amino acids, 13 fatty acids and lipids, eight sugars, and 21 other metabolites with a known and 25 with an unknown chemical structure. Heritabilities for the metabolites varied considerably from 0 to 0.85 with a mean of 0.47 (Suppl. Fig. 5). This confirms the results of Beleggia et al. (2013) that metabolite profiles in wheat grains depend highly on the environment in which they are grown.

In the principal component analysis of all 120 wheat flour samples based on metabolite profiles, the first two principal components explained 21% and 18% of the variance (Fig. 4). The samples from the location HOH were clearly separated from those from the two other locations with respect to the second principal component. In contrast, the samples from the two locations GAL and IHO were assigned to one overlapping cluster. The correlation pattern among the 104 metabolites revealed hot spots of highly correlated compounds in the metabolite profile that corresponded to a functional classification of the metabolites (data not shown). We observed that single metabolites were significantly ($P < 0.05$) associated with the assessed phenotypic characters (Fig. 5). Many metabolites were significantly ($P < 0.05$) correlated with the height/width ratio of the breads. Furthermore, we observed a cluster of five organic acids and polyols, and one unknown metabolite that was highly correlated both with the odor and the flavor of the breads.

Currently, the standard procedure in plant breeding programs is to predict adjusted entry means from SNP genotyping profiles. In our study, this corresponds to predicting adjusted entry means of bread aroma and quality characteristics from S, for which cross-validated prediction abilities between −0.29 and 0.57 (Fig. 6) were observed for the different traits. For ten of the 14 examined characters, the prediction abilities were increased, when using joined weighted relationship matrices calculated from SNP genotyping and metabolite profiles (Table 2). Only for crust hardness, dough quality, pore structure, and volume of sedimentation was the maximum prediction ability observed using only the SNP genotyping profile as predictor. Furthermore, we observed on average a decrease of the prediction ability when increasing the minimum h^2 value of a metabolite to consider it for prediction across all 14 characteristics. The prediction of the adjusted entry means of the bread aroma and quality characteristics across HOH and GAL from the adjusted entry means of the metabolites resulted in prediction abilities that were not significantly different from those observed when using the metabolite profiles from the individual locations GAL or HOH as predictors (Table 2). Only the prediction abilities for the predictions made with M_{IHO}, a location from which no bread aroma and quality characteristics were assessed, were lower.

In addition to the prediction of adjusted entry means of bread aroma and quality characteristics across multiple locations, we were interested in assessing the prediction of location specific characteristics of the 40 wheat genotypes. This was evaluated using four different approaches. The lowest prediction abilities were observed on average across all 14 bread aroma and quality characteristics for the approach A1 in which predictions were made based on SNP genotyping profiles only (Table 3) and, thus, is the baseline model. The prediction abilities were increased when using a joined weighted relationship matrix calculated from SNP and metabolite profiles (A2). On average across all traits, the highest prediction abilities for individual locations were observed for approaches A3 and A4 for which the location specific metabolite profiles were considered in the predictions. The prediction abilities observed for A3 and A4, however, were still only about 1/2 of those observed when predicting the adjusted entry means of bread aroma and quality characteristics.

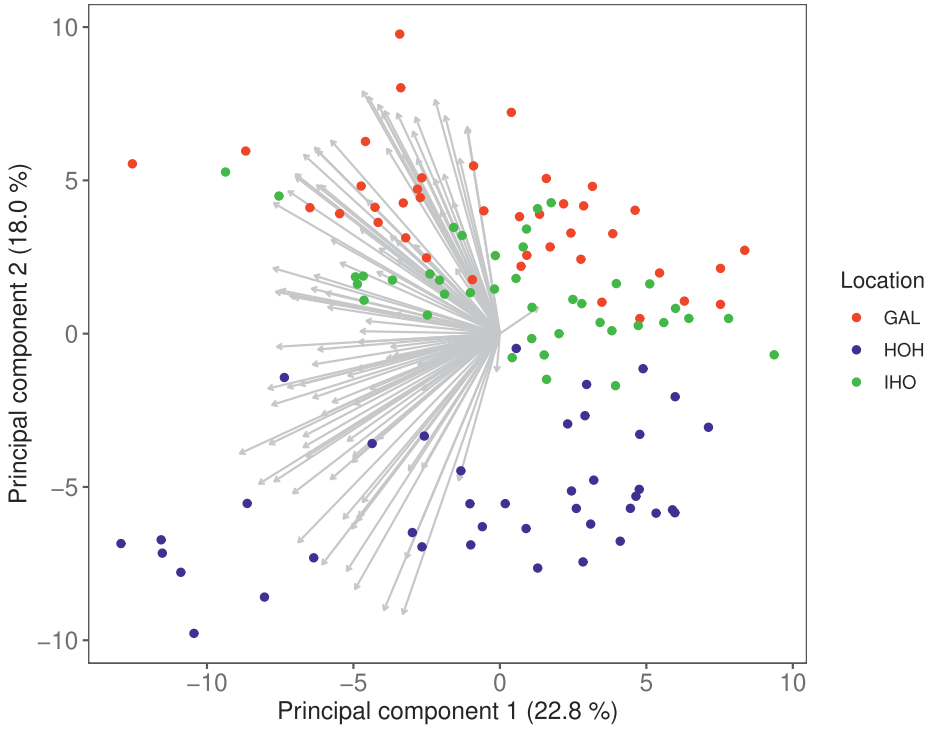

Fig. 4. Principal component (PC) analysis biplot of the metabolite profiles of 40 wheat genotypes from three locations. The arrows represent the 104 metabolites. The numbers in parentheses refer to the proportion of variance explained by the PC.

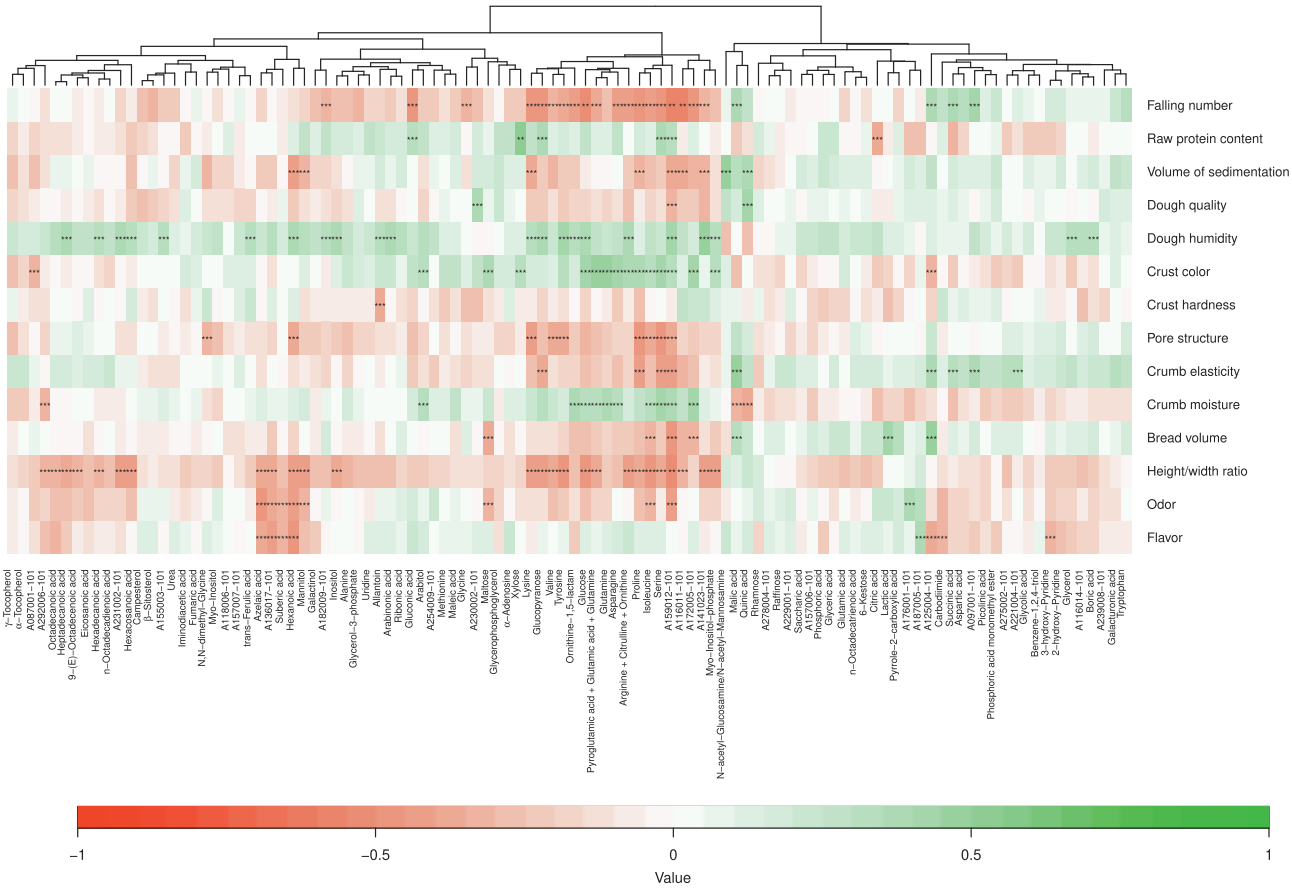

Fig. 5. Heat map of Pearson correlation coefficients calculated between all pairs of adjusted entry means of bread aroma and quality characteristics as well as the adjusted entry means of the metabolites across HOH and GAL. Correlations marked with ∗,**,*** were significantly ($P < 0.05$, 0.01, 0.001) different from 0.

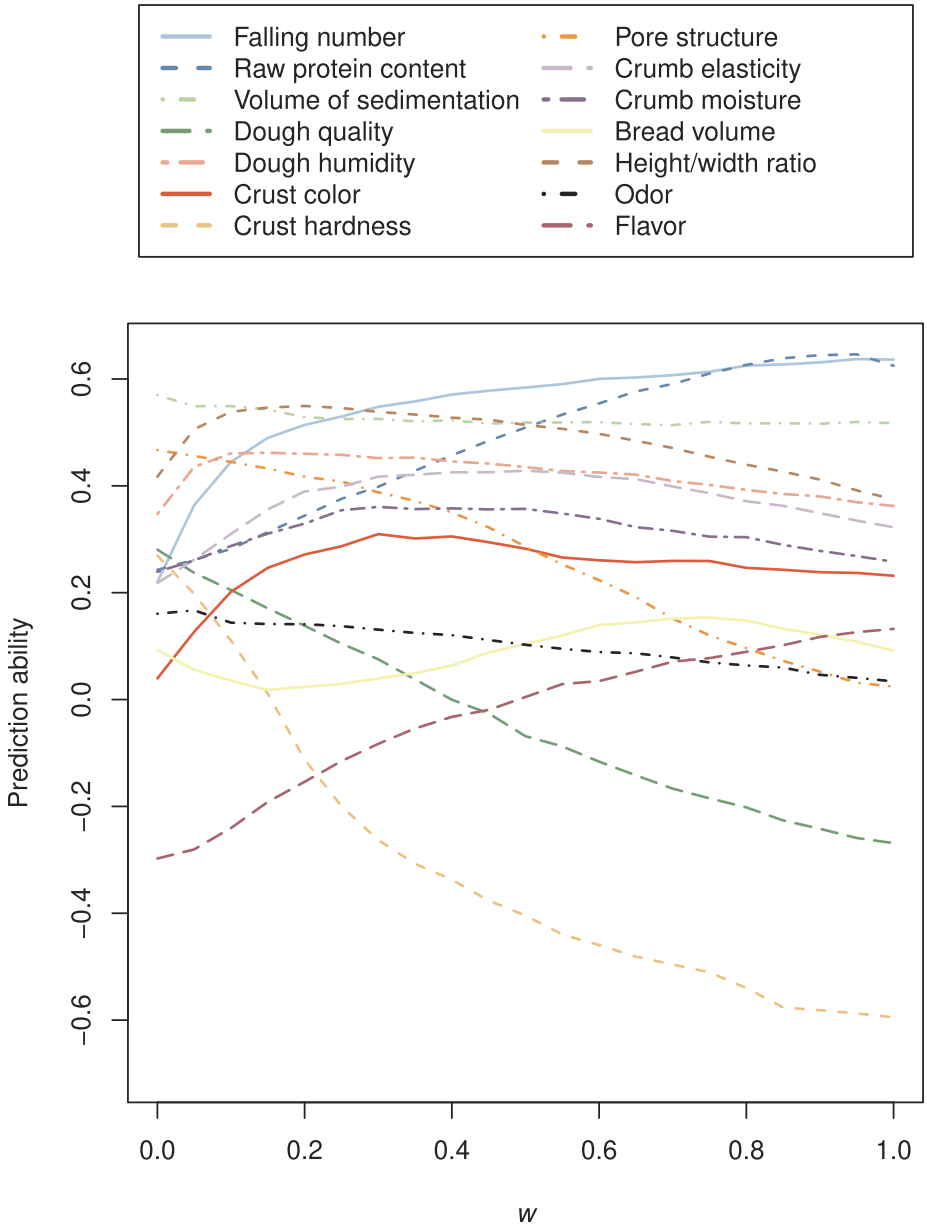

Fig. 6. Prediction abilities to predict the adjusted entry means of 14 bread aroma and quality characteristics across two locations, for 21 different joined weighted relationship matrices, where *w* is the relative weight of the metabolites compared to the SNP genotyping profiles in the joined weighted relationship matrix. The plotted values represent the medians of the prediction ability across 1000 cross-validation runs.

4. Discussion

Hundreds of wheat varieties are registered on a global scale with an annual renewal of 10–15%. During the process of breeding and registering new wheat varieties, the agronomic performance, quality characteristics such as protein content and quality, and baking characteristics (cf. Laidig et al., 2017) are considered. However, the aroma of breads, which is one of the first characteristics perceived by consumers (Pico et al., 2015), is mostly disregarded. Therefore, in our study the variation of odor and flavor in breads baked from 40 wheat genotypes was examined and the ways to improve the selection possibilities by predicting these parameters from SNP genotyping or metabolite profiles were studied.

4.1. Flavor and odor of bread are heritable characteristics

The 40 investigated wheat varieties differed considerably in all investigated characteristics, and significant genetic variances were observed for most traits (Table 1, Figs. 2 and 3). Interestingly, the breads of the 40 different wheat varieties also differed considerably in odor and flavor. The heritability h^2, which quantifies the proportion of phenotypic variation that is caused by genetic variation, was about 0.4 for both characteristics. This finding illustrates that genetics and environment influence the expression of these characteristics. The exploitation of the environmental influence for producing wheat with a more intense odor or flavor requires an understanding of the relative influence of the components of an environment on these characteristics. As this information is not available as of now and furthermore cannot be currently altered on a commercial production scale, the exploitation of the environmental influence for producing wheat with a more intense odor or flavor is limited. In contrast, choosing varieties with a genetically more intense bread odor and/or flavor is feasible and tractable along the product chain. These pioneering results for wheat confirm findings for spelt wheat, which have illustrated the potential of

Table 2
Median of prediction abilities $r_{(\hat{g},y)}$ across 1000 cross-validation runs to predict the adjusted entry means of 14 bread aroma and quality characteristics using the SNP genotyping profile S in combination with the adjusted entry means of all 104 metabolites across the field locations GAL and HOH as predictors (M) or the metabolite profiles collected for individual locations (M_{GAL}, M_{HOH}, M_{IHO}). Here, *w* is the relative weight of the metabolites compared to the SNP genotyping profiles in the joined weighted relationship matrix for which the highest prediction ability was observed in the grid search.

Trait	S&M		S& M_{GAL}		S& M_{HOH}		S& M_{IHO}	
	w	$r_{(\hat{g},y)}$	*w*	$r_{(\hat{g},y)}$	*w*	$r_{(\hat{g},y)}$	*w*	$r_{(\hat{g},y)}$
Falling number	0.95	0.64	0.30	0.53	1.00	0.66	0.35	0.28
Raw protein content	0.95	0.65	0.70	0.49	0.95	0.58	1.00	0.65
Volume of sedimentation	0.00	0.57	0.00	0.57	0.00	0.57	0.00	0.57
Dough quality	0.00	0.28	0.00	0.28	0.00	0.28	0.00	0.28
Dough humidity	0.15	0.46	0.25	0.45	0.25	0.45	0.35	0.43
Crust color	0.30	0.31	0.25	0.16	0.45	0.23	0.00	0.04
Crust hardness	0.00	0.27	0.00	0.27	0.00	0.27	0.15	0.31
Pore structure	0.00	0.47	0.00	0.47	0.25	0.52	0.00	0.47
Crumb elasticity	0.50	0.43	0.35	0.42	0.35	0.43	0.45	0.34
Crumb moisture	0.30	0.36	0.45	0.38	0.30	0.40	0.00	0.24
Bread volume	0.75	0.15	0.00	0.09	0.50	0.32	0.45	0.21
Height/width ratio	0.20	0.55	0.25	0.54	0.15	0.52	0.25	0.49
Odor	0.05	0.17	0.35	0.21	0.00	0.16	0.00	0.16
Flavor	1.00	0.13	1.00	0.27	1.00	0.09	0.00	−0.30

Table 3
Median of prediction abilities $r_{(\hat{g},y)}$ across 1000 cross-validation runs to predict genotype∗location interactions of the 14 bread aroma and quality characteristics using four different statistical approaches A1–A4. For A1, the relationship between genotypes was modeled only based on SNP genotyping profiles S. For A2, the relationship between genotypes was modeled as optimal combination of S and the adjusted entry mean of the metabolites across the locations M. For A3, the relationship between genotypes was modeled only based on S and the relatedness between samples was modeled by the sample specific metabolites. For A4, the relationship between genotypes was modeled as optimal combination of S and M and the relatedness between samples was modeled by the sample specific metabolite profiles. Here, *w* is the relative weight of the metabolites M compared to that of the SNP genotyping profiles S in the joined weighted relationship matrix. For details, see materials and methods.

Trait	A1	A2		A3	A4	
	$r_{(\hat{g},y)}$	*w*	$r_{(\hat{g},y)}$	$r_{(\hat{g},y)}$	*w*	$r_{(\hat{g},y)}$
Falling number	0.27	0.55	0.35	0.45	0.00	0.45
Raw protein content	−0.39	1.00	−0.04	0.16	0.00	0.16
Volume of sedimentation	0.17	0.15	0.22	0.43	0.00	0.43
Dough quality	−0.11	0.50	0.01	0.09	0.00	0.09
Dough humidity	0.21	0.15	0.29	0.22	0.20	0.27
Crust color	0.08	0.05	0.10	0.26	0.00	0.26
Crust hardness	−0.13	0.75	0.12	0.00	0.60	0.07
Pore structure	0.15	0.00	0.15	0.12	0.00	0.12
Crumb elasticity	0.10	0.30	0.19	0.20	0.00	0.20
Crumb moisture	0.08	0.50	0.26	0.46	0.05	0.46
Bread volume	0.11	0.25	0.14	−0.04	0.50	0.07
Height/width ratio	0.16	0.45	0.21	0.24	0.00	0.24
Odor	NA	0.85	−0.33	0.05	0.00	0.05
Flavor	−0.04	0.35	0.04	0.02	0.30	0.05

varietal choice and breeding for an improved bread aroma (Rapp et al., 2017). If in the future the selection of wheat varieties with a more intense odor and flavor becomes an objective, robust screening systems have to be elaborated, thus warranting further research.

Wheat varieties have to fulfill several dozens of criteria in order to be accepted by the farmers and the bread industry. In short, the requirements are good agronomic performance, good baking quality, which is often measured by baking volume, protein quality and content, as well as starch-related characteristics like falling number (e.g., Laidig et al., 2017). The consideration of additional selection criteria such as odor and flavor will only not impair the gain of selection for the above mentioned traits if there is no negative correlation between them. The newly investigated characteristics, bread odor and flavor, did not correlate negatively to yield or any other of the 14 investigated characters (Fig. 2, Suppl. Fig. 3). In contrast, bread odor was positively correlated with bread quality (as measured by the height/width ratio) and with flavor. This finding indicated that breeding for intense bread aroma is feasible without having a negative impact on the currently important selection criteria.

This explanation agrees with the observation that no significant mean differences in odor and flavor between old and modern wheat varieties (Fig. 3) were observed. Such differences would have been expected had negative correlations existed between odor/flavor and traits that are strongly selected in wheat breeding programs. Instead, we observed a higher variance of flavor for the group of modern wheat varieties than for the old varieties. Therewith, more aromatic breads are made out of modern rather than old wheat varieties. This finding confirms preliminary results in spelt (Rapp et al., 2017) and durum wheat (Ficco et al., 2017). Therefore, our results indicate that the opinion of bakers and consumers that today's bread is less aromatic than before is not caused by the advances made in wheat breeding for traits such as grain yield or height/width ratio (Fig. 3).

Besides the changes of the wheat varieties used for bread preparation, the changes in bread-making technology over the last decades has the potential to considerably influence the aroma of bread (Pico et al., 2015; Ficco et al., 2017). However, this was not the objective of our study and requires further research.

4.2. Combining metabolomics with genomics to predict bread aroma and quality characteristics

The breeding for bread aroma and quality characteristics is hampered by the high effort necessary to assess these traits. This restricts their assessment to late stages of the breeding process, which, in turn, leaves only little variability for selection. Accordingly, the selection of bread aroma and quality characteristics would be much facilitated if these characters could be predicted from molecular features early in the breeding process. Currently, the standard procedure in plant breeding programs is to predict adjusted entry means from SNP genotyping profiles. In our study, we have observed cross-validated prediction abilities between −0.29 and 0.57 (Fig. 6). With the exception of the values observed for odor, flavor, bread volume, and crust color, which were particularly low, these were comparable to the prediction abilities reported earlier for wheat, considering differences in the size of the calibration set (Crossa et al., 2010). Moreover, the low prediction abilities for odor and flavor might be explained as follows: both are complex characters driven by many chemical compounds present in the flour or modified during the dough preparation and baking process (Grosch & Schieberle, 1997; Starr, Bredie, & Hansen, 2013; Starr, Hansen, Petersen, & Bredie, 2015; Birch, Petersen, & Hansen, 2014; Pico et al., 2015; Ficco et al., 2017). Due to technological advances, for a few years, it is possible to assess the compounds in various tissues in a large number of samples using various techniques (Lisec et al., 2006). Such a characterization of breads has the potential to identify the chemical compounds that are causal for the odor and flavor of breads (cf. Yan et al., 2019) thus resulting in high prediction abilities. However, such approaches require baking breads, which is a limiting factor for high throughput analyses. Therefore, the prediction of bread aroma from metabolic profiles of bread is of limited utility in a plant breeding context. In contrast, the prediction of bread aroma from metabolic profiles of flour could be easily integrated in the breeding process. Therefore, in our study, GC?MS of polar soluble metabolites was used to characterize the metabolite profile of the whole grain wheat flour of 40 wheat genotypes, which were grown next to each other at three geographically divergent locations.

In the first step, we evaluated the correlation coefficients between individual metabolites and the bread aroma and quality characteristics on average across the locations. For some of the 104 determined metabolites, we found highly significant correlations with the investigated quality traits on average across the examined locations (Fig. 5). For instance, a set of metabolites negatively correlated with both falling number and height/width ratio, where falling number and height/width ratio showed a strong positive correlation (Suppl. Fig. 3) (cf. Klingler, 1995; Laidig et al., 2017). Of these metabolites, tyrosine, lysine, glucopyranose, and glucose had a heritability >0.7. Furthermore, the latter three showed the strongest negative correlation with the falling number and height/width ratio, making them interesting as potential metabolite markers.

A second remarkable finding concerns odor, flavor, and the height/width ratio, all of which correlated negatively with a set of six metabolites consisting of azelaic acid, suberic acid, hexanoic acid, galactinol, mannitol, and one unknown metabolite. This is particularly interesting because the three quality traits also correlated positively with each other (Suppl. Fig. 3). From this group, however, only mannitol had a heritability considerably greater than zero.

The assessment and quantification of single or few interesting metabolites using current GC?MS approaches, however, do not offer advantages regarding time and costs compared to a comprehensive scan as performed in the current study. Therefore, we used in analogy to the above described prediction approach based on the SNP genotyping profiles also a combination of the SNP information and all metabolites for prediction. For ten of the 14 examined characters, the prediction abilities increased when using joined weighted relationship matrices calculated from SNP genotyping and metabolite profiles compared to SNP only predictions (Table 2). This finding might be explained as follows: the metabolome information incorporates metabolic and physiological epistasis and therefore has a considerably higher prediction ability compared to SNP information, even when modeling statistical epistasis (Schrag et al., 2018). Therewith, our results suggests that for the prediction of physiologically complex traits such as odor and flavor (Grosch & Schieberle, 1997; Starr et al., 2013; Starr et al., 2015; Birch et al., 2014; Pico et al., 2015; Ficco et al., 2017), the combination of SNP and metabolome information is highly recommended.

Furthermore, when planning the integration of metabolite profiling in the practical wheat breeding program, one important aspect is the number of locations of which the wheat flour should be characterized by metabolite profiling. The adjusted entry means of the bread aroma and quality characteristics predicted from the adjusted entry means of the metabolites resulted in the same prediction abilities compared to the prediction based on metabolite profiles from only one location (Table 2). This observation indicated that the mean of bread aroma and quality traits can be well predicted when using the metabolite profiles from only one location. This finding was unexpected as we have observed a large environmental impact on the abundance of many metabolites (Suppl. Fig. 5) as was also reported for other crop species (Sprenger et al., 2018).

This environmental impact on the abundance of many metabolites became also visible in a principal component analyses (Fig. 4). The second component which explained >18% of the variance clearly separated the wheat samples from the location HOH and GAL. This separation of locations was in good accordance with the one observed for the quality traits (Fig. 1). The metabolite profiles of the wheat samples of the third location IHO were clustered together with the location GAL. This finding was unexpected as both locations are >400 km distant from each other while IHO and HOH are only 50 km away from each other. Due to the enormous labor of bread baking tests, we were unable to collect bread aroma and quality characteristics from breads baked from the flour of the IHO locations. Therefore, we could not verify whether the joint clustering of the location GAL and IHO holds true for the bread aroma and quality characteristics.

According to the above described phenomenon, that the quality traits as well as the metabolites showed a strong separation of the samples from the two locations provoked for examination in addition to the prediction ability of the adjusted entry means and the prediction ability of location specific bread aroma and quality characteristics. The prediction abilities of approaches A3 and A4 for which the location specific metabolite profiles were considered in the predictions were, on average across all traits, about fourfold the value of approaches using SNP genotyping profiles only (Table 3). This suggests that location specific metabolite profiles can boost the prediction ability of location specific effects but at the extra costs that metabolite profiles must be created for each location. Nevertheless, such approaches can be used to assess the stability of characters across locations that are expensive to evaluate such as bread aroma and quality characteristics.

4.3. Conclusions

Based on the gold standard of bread characterization in Germany, we could show that flour from different wheat varieties led to different aroma profiles of their breads. The estimation of variance components indicated that the variety, and the environment under which the variety is cultivated, influence bread aroma to about the same extent. While environmental conditions in the production of wheat can hardly be standardized, the choice of the wheat variety can be based on its potential for intensive bread aroma. Interestingly, bread aroma was not correlated with agronomic traits like grain yield or baking quality traits such as height/width ratio or bread volume. Thus, wheat varieties that combine high yield with high bread making quality as well as intensive bread aroma exist. In summary, our results indicate that wheat breeding for bread aroma is possible, especially if predictive breeding based on combinations of molecular markers and metabolite profiles of wheat flour is implemented.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest. All authors read and approved the final manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodres.2019.108748.

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