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Use of Polymorphisms in the γ-Gliadin Gene of Spelt and Wheat as a Tool for Authenticity Control†

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ABSTRACT: Partial sequencing of the γ-gliadin gene of 62 spelt and 14 soft wheat cultivars was performed. Fifty-six of the 62 spelt cultivars and 13 of the 14 soft wheat cultivars were shown to exhibit the typical spelt or soft wheat γ-gliadin sequence, respectively. Exceptions were ascribed to crossbreeding of soft wheat and spelt. Using the typical soft wheat γ-gliadin sequence, two alternative DNA-based analytical methods were developed for the detection and quantification of spelt flour "adulteration" with soft wheat. A simple and fast detection of soft wheat in spelt flours could be achieved by restriction fragment length (RFLP) analysis. In combination with lab-on-a-chip capillary gel electrophoresis (LOC-CE) the soft wheat proportion could be estimated. Heteroduplex formation served as additional confirmation for the presence of spelt besides soft wheat. Hence, RFLP-LOC-CE constitutes a perfect analysis tool for the quality control of cereal seeds and pure cultivars. A precise quantification of soft wheat "adulterations" in spelt flour down to 1% could be achieved by the developed real-time PCR method. The calibration parameters of the real-time PCR assay fulfilled the minimum performance requirements of the European Network of GMO (genetically modified organisms) Laboratories (ENGL).

KEYWORDS: *spelt "adulteration", PCR-RFLP, lab-on-a-chip capillary gel electrophoresis, multitemplate PCR, heteroduplexes, real-time PCR*

INTRODUCTION

A century ago, the hexaploid spelt (*Triticum aestivum* ssp. *spelta* (L.) Thell.) was the most important grain for breadmaking in southern Germany, Austria, and Switzerland. Since then, the free-threshing soft wheat (*Triticum aestivum* ssp. *vulgare* (Vill.) Mackey) has become prevalent. However, owing to its reported tastiness, ease of digestion, and expected greater nutritional value, demand for spelt products has risen over the past two decades, with its price often reaching twice that of comparable soft wheat products. Notably, there is no scientific evidence to spotlight spelt as an alternative for patients of celiac disease or wheat allergy.[1] Spelt has distinct agronomic properties: as a relatively undemanding crop, it has been traditionally cultivated in regions with harsh climates.[2] It can be grown on poor soils using minimal amounts of fertilizer (thus appealing to organic farmers), has high disease resistance, and assimilates nitrogen easily.[3] Spelt is more difficult to harvest mechanically owing to its long straw, which results in lodging. In addition, harvest yields are lower than those of soft wheat because the husks account for loss of up to 30%, the milling process requires an additional step to separate the husks, and spelt flour has weaker baking qualities.

To temper such disadvantageous properties, spelt has always been crossed with wheat; accordingly, a number of cultivars of spelt–wheat crosses exist.[4−7] In 1993 the spelt market was dominated by only three cultivars: Bauländer Spelz, Rouquin, and Schwabenkorn, as described by Kling.[8] Since then, many new cultivars have been bred, and others fallen out of use. Apart from that, wheat flour is sometimes added intentionally to spelt flour either to improve its baking properties or to lower the costs. Additionally, accidental cross-contamination may occur when volunteer wheat grows at low frequencies in a spelt field. According to the market intervention rules of the European Union (Commission Regulation (EC) No. 1272/2009) 7% of a total grain yield may be of species other than those indicated and still represent good agricultural practice. In milling or manufacturing, residues of wheat flour might contaminate spelt flour or spelt products, for example, when the same machinery is used for both product lines without adequate cleaning. In compliance with German food legislation, it is not allowed for spelt bread to contain >10% flour from other grains without explicit labeling.[9]

Wheat and spelt can be easily visually differentiated at early stages by traits such as glume shape and tenacity, rachis fragility, spike length, and plant height.[10] Pure grains or pure flours from wheat and spelt cultivars can also be differentiated. Ruibal-Mendieta et al.[11] used the lipid content and the lipid composition, particularly the fatty acid patterns, to distinguish between wholemeal of wheat and spelt. Recently, this method was applied to a large sample collection of spelt and wheat cultivars.[12] Cereal storage proteins are also promising tools for species differentiation. Along with their encoding genes, these proteins showed a higher degree of variability when compared to metabolic proteins. Besides electrophoretic analysis of characteristic seed storage proteins,[13−18] also reversed phase high-performance liquid chromatography/mass spectrometry (RP-HPLC/MS) methods have been developed recently.[19]

Many methods for the identification of species in cereals using polymerase chain reaction (PCR) are found in the

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Table 1. Primer and Probe Sequences Used in This Study

primer/probe	sequence 5′−3′	ref
Amp_fw	GGATCAGCGGATGTTGCTTT	this study
Amp_rv	ATGCACCACCACCCATAGAA	this study
GAG-RFLP-Fw	CAACAACAGGTGAACCCATGC	this study
GAG-RFLP-Rev	TGGACGAGAGTACCTTGACCCACC	this study
GAG15	GCAACCACAACAACAATTTTCT	5
GAG16	GATATAGTGGCAGCAGGATATG	5
GAG31	GCAGCAAGAACAACAAGAACAA	32
GAG28	CGGCGACTACGCTGGA	31
probe GAG	FAM-TACTCTCGTCCAGGGCCAGGGCATC-BHQ-1	this study

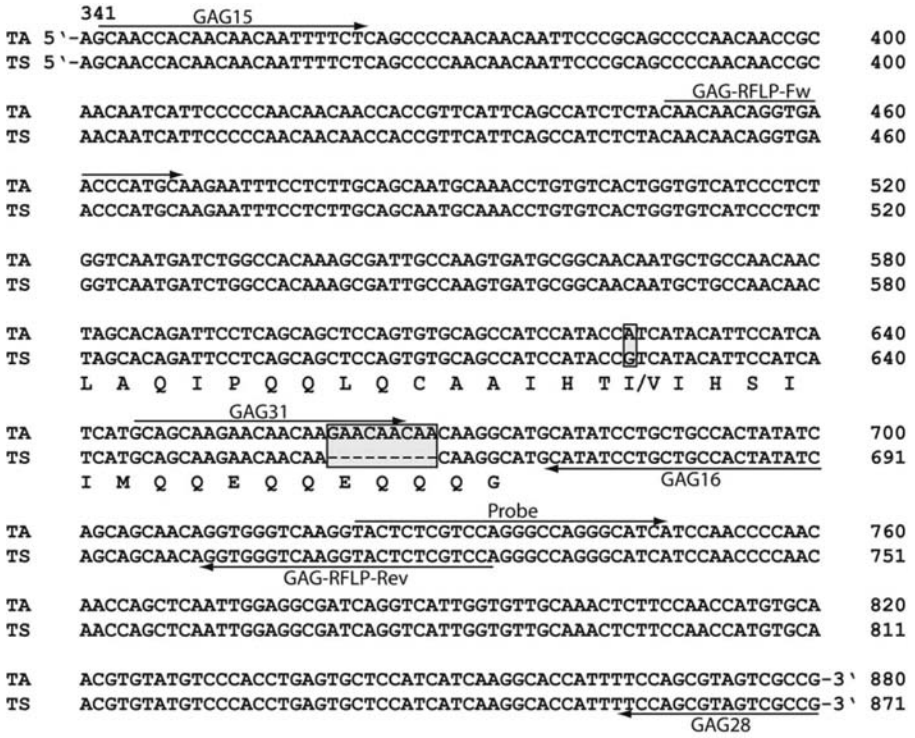

Figure 1. Sequence alignment of the γ-gliadin gene from wheat (TA, accession no. AF144104.1) and spelt (TS, accession no. AF120267.1). Primers and probes used in this work are marked by arrows. Differences in the DNA sequences are highlighted. A partial amino acid sequence is shown below the DNA sequence to show the resulting differences in the protein sequence. The numbering corresponds to the sequences given by the accession numbers.

literature. For example, PCR served as a method to detect soft wheat as a contaminant in nonwheat dietary products[20] or in durum wheat.[21−24] Ko et al.[25] focused on variable regions between the ribosomal 5S RNA (5S rRNA) genes to distinguish maize, barley, sorghum, rye, rice, oat, and wheat. The detection of the celiac disease-causing gluten-containing cereals wheat, barley, and rye has been the aim of other investigations using PCR.[26−30]

Wheat and spelt have been previously differentiated by two polymorphisms on the intronless γ-gliadin gene of the D-genome.[5,31,32] (i) A single-nucleotide polymorphism (SNP) between the wheat and the spelt sequence represents a restriction site that could be used for restriction fragment length polymorphism analysis (RFLP). (ii) A 9 bp insertion in the wheat sequence constitutes the other polymorphism enabling the design of specific PCR primers for a wheat specific PCR.

The work presented here focuses on the development of a DNA-based method for the qualitative and quantitative analysis of wheat in spelt products in routine analysis. On the basis of the differences in the γ-gliadin gene, a PCR-RFLP method and a wheat-specific real-time PCR method were developed. For qualitative detection and estimation of wheat/spelt ratios the PCR-RFLP was combined with capillary gel electrophoresis (CE) as described earlier for other species.[33−37] Heteroduplex formation served as additional confirmation for the presence of wheat. Precise quantification of even small amounts (∼1%) of wheat was performed by real-time PCR.

MATERIALS AND METHODS

Spelt and Wheat Samples. This study included 62 spelt cultivars and 14 relevant wheat cultivars (Table 1). All samples were available as complete grains and, when possible, the spelt cultivars were taken in their husks. Ancient cultivars were included, as they could potentially be used in breeding programs. All samples fulfilled the purity requirements of certified seeds, that is, at least 99%.

Wholegrain flours were milled to fine powders using a laboratory mill (Janke & Kunkel, Staufen, Germany). Authentic wheat and spelt mixtures were prepared by mixing wholegrain flours.

DNA Extraction. DNA was extracted from 200 mg of wholegrain flour weighed into a 2 mL microfuge tube. Two sterile stainless steel

Table 2. Spelt and Wheat Varieties Used for Partial Sequencing Analysis of the γ-Gliadin Gene (Primer Pair GAG15/GAG16)[a]

spelt variety	seq	spelt variety	seq	wheat variety	seq
Albin	S	Neuenegger Weißkorn	S	Akteur	W
Alkor	**W**	Oberkulmer Rotkorn*	S	Bussard	W
Altgold Rotkorn	S	Öko 10	S	Chinese Spring	**S**
Ardenne	**W**	Ostar	S	Cubus	W
Badengold	S	Ostro	S	Dekan	W
Badenkrone	S	Österr. Burgdorf	S	Herrmann	W
Badenstern	**W**	Poème	S	Impression	W
Balmegg	S	Redoute	S	Manager	W
Bauländer Spelz	S	Renval	S	Maris Hustler	W
Bregenzer Roter Spelz	S	Ressac	**W**	Mulan	W
CDCSpelt0304	S	Roter Schlegeldinkel	S	Potenzial	W
Ceralio	S	Rouquin	S	Tommi	W
Cosmos	S	Samir	S	Virtus	W
Divimar	S	Schwabenkorn	S	Winnetou	W
Ebners Rotkorn	S	Schwabenspelz	**W**		
Elsenegger	S	Sertel	S		
Emilius	**W**	Sirino	S		
EP1H.22	S	Spy	S		
Franckenkorn	S	Steiners Roter Tiroler	S		
Fuggers Babenhauser	S	Strickhof	S		
Goldir	S	Stone	S		
Hercule	S	Tauro	S		
Holstenkorn	S	Titan	S		
Hubel	S	V. Rechb. fr. Winterd.	S		
Kippenh. Roter Spelz	S	V. Rechb. br. Winterd.	S		
Kippenh. Weißer Spelz	S	Vögelers Dinkel	S		
LBA.18	S	Wag. H. W. Kolbensp.	S		
Lignee 24	S	Weißer Kolbenspelz	S		
Lueg	S	Zeiners w. Schlegeld.	S		
Müllers Gaiberger	S	Zollernspelz	S		
Neuegger Dinkel	S	Zuzger	S		

[a]The obtained sequences exhibited either the typical wheat (W) or spelt (S) sequence as given in Figure 1. Exceptions are noted in boldface.

beads ($^1/_8$ in.) were added, and the dry material was first crushed for 10 min at 30 Hz in a TissueLyser (Qiagen, Hilden, Germany). One milliliter of extraction buffer (55 mM CTAB, 1.4 M NaCl, 0.1 M Tris-HCl, 20 mM Na_2-EDTA, pH 8.0) was added and the material crushed for a further 10 min at 30 Hz in the TissueLyser. The mixture was incubated for 10 min at 65 °C in a water bath. Two hundred and fifty microliters of chloroform was added. After vortexing, the mixture was centrifuged at 14000 rpm for 10 min. Six hundred and fifty microliters of the supernatant was transferred to an EconoSpin silica membrane cartridge (Epoch Biolabs, Missouri City, TX). After centrifugation at 14000 rpm for 1 min, the run-through was discarded. The silica membrane was washed using 500 μL of washing buffer I (20 mM Tris-HCl, 1 mM Na_2-EDTA, pH 7.4) by centrifugation at 14000 rpm for 1 min. The run-through was discarded. The silica membrane was further washed with 500 μL of washing buffer II (70% ethanol, 10 mM NaCl). After centrifugation to dryness for 10 min at 14000 rpm, the silica membrane cartridge was transferred to a new 1.5 mL microfuge tube. The DNA was eluted twice with two 50 μL volumes of sterile water and centrifugation for 1 min at 7000 rpm. DNA was stored at −20 °C until usage. The purity of DNA extracts was evaluated by the 260 nm/280 nm extinction ratio. The concentration of DNA extracts was measured fluorimetrically using SYBR Green I (Invitrogen, Eugene, OR) and a calibrator (pBluescript II SK(−), Fermentas GmbH, St. Leon-Rot, Germany), 20 ng/μL). DNA extracts were adjusted with water to a final concentration of 20 ng/μL.

Qualitative PCR. One microliter (~20 ng) of the DNA extracts was used for PCR. PCR reactions were carried out in a final volume of 25 μL. The PCR reaction mix contained 10× Mg^{2+}-free F-510 DyNAzyme buffer (final concentration = 10 mM Tris-HCl, pH 8.8, at 25 °C, 50 mM KCl, 0.1% Triton X-100, Finnzymes Oy, Espoo, Finland), 3 mM $MgCl_2$ (Finnzymes Oy), 5% DMSO (Carl Roth GmbH, Karlsruhe, Germany), dNTPs 200 μM each (Bioline GmbH, Luckenwalde, Germany), primers 1 μM each (Invitrogen, Darmstadt, Grmany), and 1 U DyNAzyme EXT DNA Polymerase (Finnzymes Oy). PCR conditions were 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 64 °C for 60 s, 72 °C for 80 s, and 1 cycle of 72 °C for 10 min before a hold at 4 °C. PCR amplification used a T3 Thermocycler (Biometra, Göttingen, Germany). Primer sequences are given in Table 1, and the hybridization sites of the primers are shown in Figure 1.

The general ability of the extracted DNA to be amplified was assessed by a qualitative PCR with the primer pair Amp-fw and Amp-rv targeting the high-copy number 18S RNA gene and enclosing an amplicon of 236 bp.

Electrophoresis. PCR products were mixed with loading buffer (10×, final concentration = 5% v/v glycerine, 0.025% w/v bromophenol blue, 0.025% w/v xylene cyanol) and separated on 2% agarose gels in TAE-buffer (40 mM Tris-acetate, pH 8.2, 2 mM EDTA) at 150 V followed by ethidium bromide staining (0.1%) and visualization under UV light.

Real-Time PCR. Wheat-specific PCR used the wheat-specific forward primer GAG 31 and the reverse primer GAG 28 described by Von Büren.[31,32] The software Beacon Designer (PREMIER Biosoft International) was used to design the TaqMan probe GAG to hybridize on the same strand as the wheat-specific forward primer GAG 31 (Table 1). Real-time PCR reactions used a capillary LightCycler 2.0 (software version 4.05) and reagents (LightCycler TaqMan Master Kit) in accordance with the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Reactions used 100 ng of template DNA in a total reaction volume

of 20 μL, 200 nM of each primer, and 700 nM of the probe. Cycling conditions were as follows: initial denaturation at 95 °C for 10 min, 45 cycles of denaturation for 10 s at 95 °C, annealing for 20 s at 64 °C, and elongation at 72 °C for 1 s. After a final cooling to 40 °C, the run was complete. Fluorescence was measured once per cycle at 530 nm during the elongation step of each cycle. For calibration, flour mixtures of spelt cultivar Franckenkorn and wheat cultivar Akteur with wheat proportions of 100, 50, 25, 12.5, 6.3, 3.1, 1.6, and 0.8% were extracted and served as calibrators to obtain the regression curve. Calibration was carried out in triplicates. In addition, two controls were measured routinely: a no-template control (NTC: water) as a test for contamination and a pure spelt sample as a negative control.

PCR-RFLP. For PCR-RFLP experiments, universal primers were designed to amplify a 278 bp spelt product and a 287 bp soft wheat product containing the 9 bp insertion (Figure 1). The design of primers, calculation of annealing temperatures, and sequence alignments were carried out using GENtle software version 2004 (by Magnus Manske, University of Cologne, Germany). The PCR was performed using the primer pair GAG-RFLP-Fw and GAG-RFLP-Rev as described above. PCR products were analyzed by agarose gel electrophoresis. The restriction enzyme *Hpy*CH4III was obtained from New England Biolabs (Ipswich, MA) and used with the supplied buffer, NEB buffer 4. *Hpy*CH4III has a temperature optimum of 37 °C and can easily be heat inactivated. PCR products were digested using 2.5 U of *Hpy*CH4III with 1 μL of NEB buffer 4 for 8.5 μL of PCR reaction mix at 37 °C for 3.5 h in a final reaction volume of 10 μL. The restriction enzyme was afterward heat inactivated for 30 min at 80 °C.

LOC-CE. All analyses were carried out using the Bio-Rad Experion System and the 1 K Reagents Kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Estimates of fragment size and quantity used the manufacturer's software and were based on a comparison with the sizing standard supplied with the kit. Quantities were calculated as relative percentage of the areas of all product signals detected (100% method).

Sequence Analysis. PCR was performed using the primer pair GAG 15 and GAG 16 reported by Von Büren et al.[5] using the conditions for qualitative PCR as described above. PCR products were purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, CA) according to the manufacturer's instructions. Preparations for sequencing were carried out using BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Carlsbad, CA) and primer GAG 15 in one direction. Sequencing was carried out by Analytical Services North GmbH, Hamburg, Germany.

RESULTS AND DISCUSSION

The DNA extraction method used was an in-house standard protocol based on the German official collection of test methods in accordance with Article 64 of the German Food Act (§64 LFGB), in combination with the use of silica membrane cartridges that was adapted to the grain matrix.[38] DNA was extracted from wheat and spelt in similar ranges, resulting in yields between 30 and 80 ng/μL of highly pure DNA from 200 mg of starting material ($R_{260\ nm/280\ nm}$ = 1.9−2.0). The ability of all DNA samples to be amplified was confirmed by PCR using the universal primer pair Amp-fw and Amp-rv (Table 1).

As described by Von Büren et al.[5] the sequences of the γ-gliadin genes of wheat and spelt differ in two polymorphisms. Sequence alignments of the γ-gliadin gene of the spelt variety Oberkulmer Rotkorn (accession no. AF120267.1) and wheat variety Forno (accession no. AF144104.1) are given in Figure 1. At position 625 a SNP (A/G) could be observed resulting in an isoleucine (ATC)/valine (GTC) exchange in the translated protein sequence. Additionally, this SNP leads to the presence of a *Hpy*CH4III restriction site (ACNGT) in the spelt sequence. Thirty-five nucleotides upstream, the wheat sequence contains a 9 bp insertion, which results in three additional amino acids (EQQ) in the peptide sequence. Both differences could be used for the DNA-based differentiation of wheat and spelt.[5,31,32] Partial sequencing of the γ-gliadin genes of all 62 spelt and 14 soft wheat cultivars under study with primer pair GAG 15/GAG 16[5] showed unambiguously either the complete wheat or the complete spelt sequence (Table 2 and Figure 1). Neither a combination of the spelt-specific *Hpy*CH4III restriction site with the wheat-specific 9 bp insertion nor any additional polymorphism could be observed. This indicated that the γ-gliadin gene of the D-genome was highly conserved.

The six spelt cultivars Alkor, Ardenne, Badenstern, Emilius, Ressac, and Schwabenspelz showed the wheat typical sequence of the γ-gliadin gene of the D-genome. Cultivar Alkor is known to be a spelt−wheat cross (Altgold × Avalon)[2] and is cultivated under a special biologic−dynamic agricultural practice and marketed accordingly.[39] Spelt market experts estimate the market share below 1%.[40] In the case of the Belgian cultivars Ardenne and Ressac, also a wheat crossbreed in the parental line is known (Ardenne = Virtus × Ligneé24; Ressac = (Rouquin × 1391.4) × Rouquin).[41,42] No pedigree information was available for Badenstern, Emilius, and Schwabenspelz, but a wheat crossbreed is supposable. The origin of cultivar Emilius is the Landessaatzuchtanstalt Baden-Württemberg (Stuttgart, Germany), and it has never entered the market. Cultivar Schwabenspelz (ZG Raiffeisen eG, Karlsruhe, Germany) is for reasons of low demand not available on the market anymore. The market share of the new cultivar Badenstern (ZG Raiffeisen eG, Karlsruhe, Germany) will be represented by the seed production areas published annually by the German federal seed authority. It had not been available before 2010.

The wheat cultivar Chinese Spring showed the spelt typical sequence of the γ-gliadin gene of the D-genome in accordance with Von Büren's results.[5] Cultivar Chinese Spring is an old Chinese landrace and is used as a model organism of the wheat genome sequencing project as large genetic stocks and aneuploid lines are available.[43] Apart from this scientific use, the cultivar has no economical relevance.

As expected, PCR amplification of spelt and wheat DNA using primers GAG-RFLP-Fw and GAG-RFLP-Rev flanking the 9 bp insertion resulted in fragments of 287 bp (wheat) and 278 bp (spelt), respectively. LOC analysis confirmed the presence of the 9 bp insertion (Figure 2), although the small difference in fragment size was not readily resolved by LOC (lanes 1 and 2). The amplified spelt and wheat fragments were differentiated by restriction digest using *Hpy*CH4III, resulting in unique spelt-derived fragments of 101 and 177 bp (lane 3). Heteroduplexes were observed in mixed PCR products presumably resulting from the 9 bp insertion/deletion (Figure 2, lanes 4 and 5, and Figure 3). Heteroduplexes were not cleaved by *Hpy*CH4III. This heteroduplex formation can be useful as additional evidence for the presence of wheat alongside spelt in one sample. However, quantitative analysis revealed less heteroduplex formation than anticipated by theoretical considerations according to the Hardy−Weinberg law[37,44] as can be seen in Figure 4 (open circles vs dotted lines). Consequently, the wheat homoduplex proportion showed a less sigmoidal progression (solid circles vs dashed curve). Using this approach we found that standard deviations for <10% adulteration were typically high, making an accurate quantification of single-digit percentages of wheat in spelt flours impossible. The detection limit was roughly estimated to be 5%.

Quantification by real-time PCR can be achieved by a standard curve through amplification of known amounts or

Figure 2. Virtual gel image of PCR-RFLP experiments using DNA from pure wheat (1) or spelt (2 and 3) flour or a mixture of spelt and wheat flour (4 and 5) as template. Lanes: L, ladder with sizes in base pairs; 1, 287 bp wheat amplicon before restriction analysis; 2, 278 bp spelt amplicon before restriction analysis; 3, *Hpy*CH4III restriction digested spelt amplicon fragments (lower band, 101 bp; upper band, 177 bp); 4, amplicons from mixed template amplification of wheat and spelt; 5, like 4, after restriction digest with *Hpy*CH4III. Heteroduplexes are indicated by arrows.

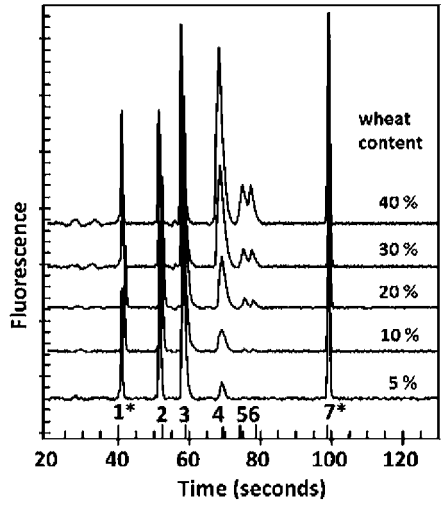

Figure 3. Electropherographs of PCR-RFLP experiments using DNA from flour mixtures containing 5−40% wheat. PCR products were subjected to the wheat-specific restriction digest and were analyzed by LOC-CE. Peaks: 1*, lower marker; 2, 101 bp restriction fragment of the spelt amplicon; 3, 177 bp restriction fragment of the spelt amplicon; 4, undigested 287 bp wheat amplicon; 5 and 6, heteroduplexes; 7*, upper marker.

known percentages of the target DNA and plotting the sample C_T values against this curve.[45] For external calibration, mixtures of wholegrain wheat flour from the cultivar Akteur and wholegrain Franckenkorn spelt flour were prepared. In unknown samples wheat cultivars other than Akteur could be present. For a reliable quantification it is therefore essential to verify that the γ-gliadin gene copy number of all wheat cultivars is the same. An example is given in Figure 5. As can be seen, C_T values did not differ between samples, confirming an identical gene copy number in all wheat cultivars. It should be noted that the six spelt cultivars that carry the wheat-specific DNA sequence exhibit the same copy number.

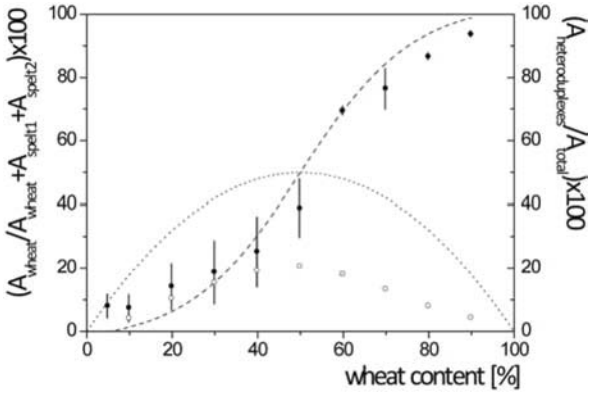

Figure 4. Comparison of the measured percentage of the heteroduplexes (right *y*-axis, open circles) and the wheat homoduplex (left *y*-axis, solid circles) with the theoretical percentages according to the Hardy−Weinberg law (dotted and dashed lines, respectively). Error bars represent the standard deviation.

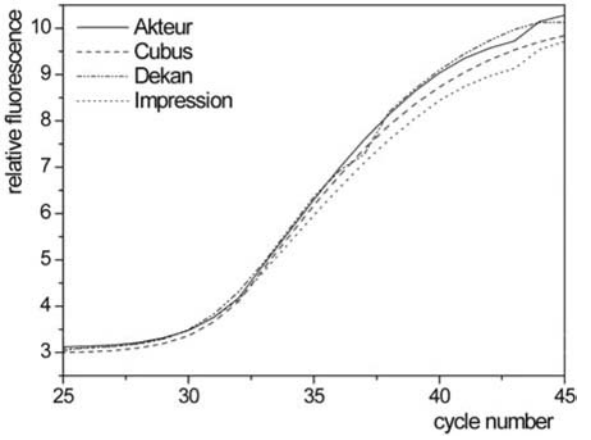

Figure 5. Real-time PCR experiments using 100 ng of wheat DNA and the wheat-specific probe detection system.

For calibration, flour mixtures of wheat and spelt were prepared. DNA was extracted directly from these flour mixtures. Notably, wheat and spelt have similar genome sizes and are both hexaploid with the genomes AABBDD. Wheat proportions in the flour mixtures ranged between 0.8 and 100%. DNA was extracted directly from these flour mixtures and therefore represented DNA proportions as well as the matrix effects. The selection of concentrations mentioned above resulted in equidistant calibration points on the logarithmic scale. Calibration was carried out in triplicate, and homogeneity of variance was achieved over the whole calibration range. Reaction curves and the resulting calibration curve are given in Figure 6. This calibration approach directly displayed weight percentages and did not have to be converted from copy number to weight.

The LOQ depends primarily on genome size, copy number, and amount of template DNA and was calculated theoretically with 0.7% for wheat by Hübner et al. (LOQ: 36 gene copies, 500 ng of template DNA/100 μL reaction mix).[46] These findings are well in line with the lowest point of the linear working range of 0.8% of the presented calibration curve. For the evaluation of calibration parameters the "minimum performance requirements for analytical methods of genetically modified organisms (GMO) testing" published by ENGL[47] were used. The observed values for efficiency (E = 114.0%),

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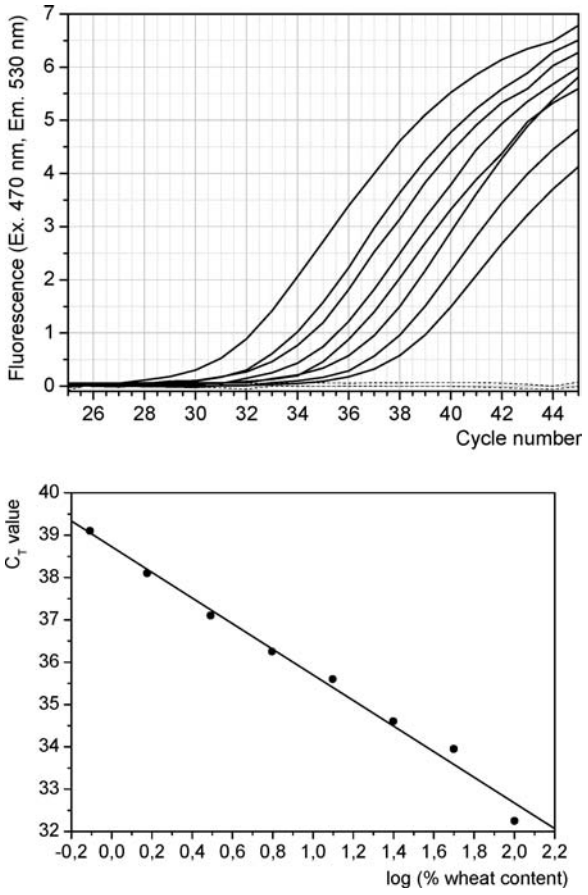

Figure 6. Matrix calibration using DNA from flour mixtures containing 0.8−100% wheat: (top) reaction curves observed with the wheat specific probe detection system; (bottom) resulting calibration curve with E = 114.0% (m = −3.026) and R^2 = 0.994.

dynamic range (0.8−100%), and R^2 coefficient (R^2 = 0.99) met these given requirements.

To check the applicability and the relevance of the presented method in the determination of wheat admixtures in spelt flours and spelt baking premixes, 30 samples of spelt flour and spelt baking premixes were purchased all over Germany in autumn 2009 and were analyzed using this real-time PCR assay.[48] In about one-third of the samples, the measured wheat content was in the single-digit percentage range, or <1%. The second third showed wheat contents of around the legal limit of 10%, and the rest indicated remarkably high amounts of up to 70%. In these cases the possibility of a false-positive detection of one of the six spelt varieties (Alkor, Ardenne, Badenstern, Emilius, Ressac, and Schwabenspelz) carrying the wheat sequence motifs had to be taken into consideration.

In conclusion, the presented work provides an update to currently available spelt cultivars and their differentiability from wheat cultivars based on the γ-gliadin gene. The stability of the copy number of the γ-gliadin gene of the D-genome between and among cultivars of wheat and crossbreeds of wheat and spelt carrying the wheat typical sequence was demonstrated. This fulfilled a major prerequisite for accurate quantification based on this trait. The combined interpretation of the sequencing results, real-time PCR measurements, and RFLP data based on the SNP on the γ-gliadin gene of the D-genome demonstrated, in accordance with Von Büren, that there were no heterozygous cultivars. However, some exceptions among the spelt cultivars carrying the wheat typical sequence were shown for the first time. Hence, for a comprehensive interpretation of analytical data, it is mandatory to identify cultivars that constitute exceptions that cannot be covered by a certain method. The PCR-RFLP-CE assay allowed the differentiation of pure cultivars. The quantitative real-time PCR assay met the calibration requirements according to ENGL and enables quantification in the range of legal limits. Both assays can be applied in routine analysis.

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DEDICATION

†Dedicated to Prof. Helmut Simon on the occasion of his 85th birthday.

ABBREVIATIONS USED

CE, capillary gel electrophoresis; ENGL, European Network of GMO Laboratories (ENGL); GMO, genetically modified organism; LOC, lab-on-a-chip; LOQ, limit of quantification; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RP-HPLC-MS, reversed phase high-performance liquid chromatography−mass spectrometry; rRNA, ribosomal RNA; SNP, single nucleotide polymorphism.

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