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Alkylresorcinol composition allows the differentiation of *Triticum* spp. having different degrees of ploidy

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ABSTRACT

Total alkylresorcinol (AR) content and homologue composition were assessed in whole grain flours of 15 varieties each of bread wheat, durum, spelt, emmer, and einkorn grown in four different environments. Bread wheat (761 ± 92 μg/g DM) and spelt (743 ± 57 μg/g) belonging to the hexaploid species showed higher AR concentrations than the tetraploid durum (654 ± 48 μg/g, $p < 0.05$), while the concentrations found in the diploid einkorn (737 ± 91 μg/g) and the tetraploid emmer (697 ± 94 μg/g) did not significantly differ from the other species. The AR content showed a remarkable heritability and, thus, seemed to be mainly determined by genetic factors. If ARs were assumed to be deposited within a specific AR-rich layer of the kernel, AR levels of all varieties would easily surpass their minimal inhibitory concentrations against fungal pathogens within this barrier layer. Although the AR carrying a C21:0 side chain was the main homologue in all species, the levels of all AR homologues and their relative composition significantly differed between hexaploid (bread wheat and spelt), tetraploid (durum and emmer) and diploid (einkorn) species. Consequently, a clear-cut differentiation of *Triticum* species and derived whole grain flours according to their degrees of ploidy was established based on concentrations of saturated C_{17}-, C_{19}-, C_{21}-, C_{23}-, and C_{25}-substituted ARs.

1. Introduction

Alkylresorcinols (ARs) are amphiphilic phenolic lipids carrying a 1,3-dihydroxybenzene (resorcinol) structure and an odd-numbered, mainly saturated alkyl side chain with up to 27 carbon atoms (Kozubek and Tyman, 1999). The plant source with an abundant amount of ARs is cashew nut (*Anacardium occidentale* L.), and in particular, its shell oil containing up to 20% of phenolic lipids, which is a by-product of cashew nut processing (Kozubek and Tyman, 1999). Furthermore, ARs were found in several cereals, mango (*Mangifera indica* L.) peels and pulp (Knödler et al., 2009), peas (*Pisum sativum* L.) (Zarnowski and Kozubek, 1999), ginkgo (*Ginkgo biloba* L.) pulp and leaves (Zarnowska et al., 2000), and Brazilian pepper (*Schinus terebinthifolius* Raddi) (Skopp et al., 1987), though the homologue pattern differs between the species. Among these, cereal grains contained the highest amounts (Ross et al., 2003), in particular within the intermediate layers of the kernel, *i.e.* the inner pericarp, the hyaline layer, and the testa (Landberg et al., 2008). Since these layers are mostly removed during common flour production, high levels are only found in whole grain flours. Consequently, ARs and their metabolites were established as potential biomarkers of whole grain intake (Ross et al., 2004). The most abundant ARs present in cereals are homologues with saturated C_{17}, C_{19}, C_{21}, C_{23}, and C_{25} side chains as shown in Fig. 1 (Ross et al., 2003).

Besides serving as a biomarker for whole grain intake, cell culture studies suggest an association of ARs with anti-mutagenic properties (Gasiorowski et al., 1996), the prevention of colon cancer (Zhu et al., 2011), and anti-inflammatory effects (Knödler et al., 2008). Moreover, an inhibitory activity of ARs against pathogenic fungi such as *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium*

Abbreviations: AR, alkylresorcinol; C17:0, 5-heptadecylresorcinol; C19:0, 5-nonadecylresorcinol; C21:0, 5-heneicosylresorcinol; C23:0, 5-tricosylresorcinol; C25:0, 5-pentacosylresorcinol; HCA, hierarchical cluster analysis; MeOH, methanol; MTBE, methyl *tert*-butyl ether; PCA, principle component analysis; PC, principal component; TKW, thousand kernel weight.

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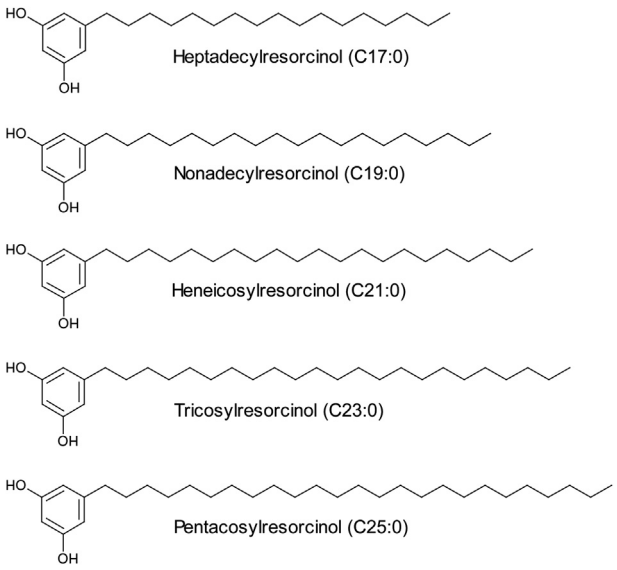

Fig. 1. Structure of major alkylresorcinols (ARs) in *Triticum*.

expansum, and *Fusarium graminearum* has been demonstrated (García et al., 1997; Dey et al., 2013; Ciccoritti et al., 2015).

When evaluating the potential bioactivity of an AR source, the determination of the homologue composition of the ARs is crucial, since their bioactivity has been hypothesized to depend on the structure of the alk(en)yl side chain (Ciccoritti et al., 2015; Stasiuk and Kozubek, 2010). Furthermore, various studies reported the AR homologue composition to be a useful parameter for the chemotaxonomic discrimination of different *Triticum* species (Chen et al., 2004; Landberg et al., 2006; Knödler et al., 2010). The C17:0/C21:0 ratio has been proposed to allow the differentiation of bread wheat (*Triticum aestivum* L.) and durum (*Triticum durum* Desf.) (Landberg et al., 2006; Knödler et al., 2010). However, further data on other cultivated wheat species is still incomplete or even unavailable. Cultivated *Triticum* species are classified into hulled and free-threshing ('naked') forms. Among the latter, bread and durum wheat are the most important *Triticum* species cultivated worldwide (Shewry, 2009). However, during the past years, underutilized hulled wheat species such as spelt (*Triticum spelta* L.), emmer (*Triticum dicoccum* Schrank), and einkorn (*Triticum monococcum* L.) gained an increased interest by consumers, especially in Germany (Miedaner and Longin, 2012). Interestingly, these species differ by three ploidy levels: diploid (einkorn), tetraploid (durum and emmer), and hexaploid (bread wheat and spelt).

In the present study, total AR contents and relative AR compositions in whole grain flours of diverse cultivated wheat species were investigated in order to elucidate intra- and interspecies differences in their AR profiles, which may be useful for the differentiation of wheat flours and other products. Furthermore, the study aimed at evaluating whether there is a relationship between the AR homologue pattern and the degree of ploidy in *Triticum* spp.

2. Materials and methods

2.1. Plant material and sample preparation

15 varieties each of bread wheat, spelt, durum, emmer, and einkorn were grown on four sites within Germany (Stuttgart-Hohenheim, Oberer Lindenhof (St. Johann), Seligenstadt, and Eckartsweier) and harvested in 2013. Cereal samples assessed in the present study were produced by the State Plant Breeding Institute (University of Hohenheim, Stuttgart, Germany) in separate but adjacent trials with two replicates for each species. After harvest, spelt, emmer, and einkorn samples were dehulled and cleaned with a laboratory seed cleaner (Samatec-Roeber, Bad Oeynhausen, Germany) in order to remove hulls, straw, and damaged kernels. Thousand kernel weight (TKW) was evaluated gravimetrically by weighing three aliquots of 100 kernels per variety. Kernel length and width were determined using a Marvin seed analyzer (GTA Sensorik, Neubrandenburg, Germany). The kernel volume was calculated as revolving ellipsoid, considering the kernel length and width. TKW, kernel length, width, and volume are provided in the Supplemental data. For chemical analyses, the kernels of two biological replicates from the same growing site were combined and milled to a particle size of $\leq$0.5 mm applying a laboratory mill ZM1 (Retsch, Haan, Germany). Dry matter content of the flours was gravimetrically determined using an infrared moisture analyzer MA 40 (Sartorius, Göttingen, Germany). Whole grain flour was stored at −20 °C until further analyses.

2.2. Chemicals

Methanol (MeOH), methyl *tert*-butyl ether (MTBE), and ammonium acetate were from VWR International (Darmstadt, Germany). Acetone was obtained from Merck (Darmstadt, Germany). The authentic reference standards 5-heptadecylresorcinol (C17:0), 5-nonadecylresorcinol (C19:0), 5-heneicosylresorcinol (C21:0), 5-tricosylresorcinol (C23:0), and 5-pentacosylresorcinol (C25:0) with purity of 95% were purchased from ReseaChem (Burgdorf, Switzerland). Purified water was prepared with a Sartorius arium 611 Ultrapure Water System (Sartorius, Göttingen, Germany).

2.3. Quantitation of ARs in wheat flour

The extraction of ARs was carried out according to a method reported previously (Ziegler et al., 2015). Briefly, an aliquot of 0.7 g whole grain flour was extracted with 2 mL of acetone using an Ultra Turrax T-25 homogenizer (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 1750 × *g* for 2 min (Heraeus Labofuge 400R, Thermo Fisher Scientific, Osterode, Germany) to separate the liquid phase from the solids. The remaining solids were re-extracted twice with 1.5 mL of acetone as described above. The combined acetone extracts were evaporated under a gentle stream of nitrogen, and stored at −80 °C until HPLC analysis.

The dried extracts were re-dissolved in 0.5 mL of MeOH/MTBE (1:1, v/v) and filtered through a 0.45 μm PTFE-Filter (Macherey–Nagel, Düren, Germany) into amber glass vials. HPLC-DAD analyses were performed using a Waters separation module 2695 with a Waters 2996 UV/vis photodiode array detector (Waters, Milford, MA, USA). The HPLC system was equipped with an RP-C18 column (Phenomenex Kinetex™, 250 × 4.6 mm i.d., 5 μm particle size, 100 Å porosity core–shell particles). The composition of the mobile phase and the elution program were reported previously (Ziegler et al., 2015). UV/vis absorption spectra were recorded in the range of 220–700 nm. ARs were monitored at a detection wavelength of 275 nm. Quantitation was performed using linear external calibration curves (1–600 mg/L) of the above mentioned authentic reference standards. Total AR concentration was expressed as the sum of the five individual homologues (C17:0-C25:0, Fig. 1).

2.4. Statistical analysis

All chemical analyses were carried out in duplicate. Correlations (Pearson correlation coefficient), means, standard deviations, ANOVA, and Tukey's HSD test as well as heritability were calculated