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The n-alkylresorcinols in durum wheat: genotypic and environmental variability

Ciccoritti Roberto^{1,2}, Silvia Bellato¹, Viviana Frate¹, Francesca Nocente¹

¹ CRA-QCE Roma, Italy

² Università Campus Bio-Medico di Roma, Roma, Italy

Abstract. The polyphenols commonly present in some cereal grains (*Triticum* species, rye and barley, mainly) include the 5-n-alkylresorcinols, alternatively called alkylresorcinols (ARs). The major AR in cereal grain contains the saturated chain with an odd number of carbon atoms in the range 17-25. These polyketides exhibit a wide spectrum of biological activities (antimicrobial, interaction with proteins, biological membranes, and enzymatic activities) which may be associated with their amphiphilic structure. Content and composition of ARs are affected by several factors such as plant species, cultivar, organ, physiological stage and environment.

The aim of the present study was to evaluate AR variability (content and homologous composition) by GC-MS of fifteen cultivars of durum wheat grown in three Italian locations (Jesi, Montelibretti and Foggia) during 2009 and 2010.

The environment (E) and the genotype (G), as well as their interaction (GxE), appeared to significantly influence the AR content in the durum wheat grains. On average, the analysed genotypes showed a variability range from 173.9 to 415.2 µg/g (dry matter, DM) and revealed similar composition of AR homologues with a high proportion of higher chain length C21:0, C23:0 and C25:0. Finally, in the present study the potential antifungal activity of ARs extracted from durum wheat against four different *Fusarium* species was also described.

Keywords. Durum wheat – Alkylresorcinols – Antifungal activity – Functional food .

Les n-alkylrésorcinols chez le blé dur : variabilité génotypique et environnementale

Résumé. Les polyphénols présents couramment dans les grains de certaines céréales (l'espèce *Triticum*, le seigle et l'orge, principalement) incluent les 5-n-alkylrésorcinols, connus aussi comme alkylrésorcinols (ARs). Le principal AR dans le grain de céréale contient la chaîne saturée avec un nombre impair d'atomes de carbone allant de 17 à 25. Ces polykétides ont plusieurs fonctions biologiques (activité antimicrobienne, interaction avec les protéines, membranes biologiques et activités enzymatiques) qui peuvent être associées à leur structure amphiphile. Le contenu et la composition des ARs sont influencés par de nombreux facteurs tels que l'espèce végétale, le cultivar, l'organe, le stade physiologique et l'environnement.

Le but de cette étude était d'évaluer la variabilité des AR (teneur et composition homologue) par GC-MS de quinze variétés de blé dur cultivées dans trois différents endroits en Italie (Jesi, Montelibretti et Foggia) en 2009 et 2010.

L'environnement (E) et le génotype (G), ainsi que leur interaction (GxE), semblent influencer de manière significative la teneur en AR dans les grains de blé dur. En moyenne, les génotypes analysés ont montré une variabilité allant de 173,9 à 415,2 mg/g (matière sèche, MS) et ont révélé une composition similaire des homologues d'AR avec une forte proportion de la longueur de chaîne supérieure C21: 0, C23: 0 et C25: 0. Enfin, l'activité antifongique potentielle des ARs extraits de blé dur contre quatre espèces différentes de *Fusarium* a également été explorée.

Mots-clés. Blé dur – Alkylrésorcinols – Activité antifongique – Aliments fonctionnels.

I – Introduction

Wheat is a major crop and one of the most important staple foods of the human diet. In recent years, research has shown that whole grain consumption was associated with significant health benefits in the management of important chronic diseases (Jacobs *et al.*, 2004; Riccioni *et al.*, 2012; de Munter *et al.*, 2007; Aune *et al.*, 2011). Current consumer demand for healthier foods has led to an increased focus on the characterization of health-beneficial compounds and their contents in whole grain. Among health promoting phytochemicals residing in whole grain, phenolic lipids have gained interdisciplinary interest in many scientific research areas as they have antioxidant properties and biological activity in prevention of cardiovascular diseases and cancer. Phenolic lipids are synthesized both during normal development and in response to stress conditions and their content is affected by several factors such as plant species, cultivar, organ, physiological stage and environment (soil, agronomy and climate) (Carbone *et al.*, 2011; Yu *et al.*, 2004). The polyphenols commonly present in the cereal grains include the 5-*n*-alkylresorcinols, alternatively called alkylresorcinols (ARs) which belong to an extensive family of bioactive compounds, widely distributed in plants, fungi and bacteria (Kozubek and Tyman, 1999; Ross *et al.*, 2003).

5-*n*-alkylresorcinols are characterized by two hydroxyl groups at positions C1 and C3 of the aromatic ring, with an odd number of saturated alkyl chains of different lengths (C15:0, C17:0, C19:0, C21:0, C23:0, C25:0) at the C5 position of the benzene ring. The amphiphilic nature of their structure could be responsible for their ability to interact with biological membranes and related with their biological properties (antimicrobial, cytotoxic, antioxidant, antitumor activity etc.) (Kozubek and Tyman, 1999; Ross *et al.*, 2003, Linko *et al.*, 2005). Among cereals, wheat, rye and triticale contain high levels of these compounds which occur only in an intermediate layer of the caryopsis, including the hyaline layer, inner pericarp, and testa and are not detected in other parts of the grain (Landsberg *et al.*, 2008). Due to their peripheral location and biosynthesis specifically during seedling stage, alkylresorcinols and their derivatives are thought to serve important roles as phytoanticipins and allelochemicals, although direct evidence is still somewhat lacking (Suzuki and Yamaguchi, 1998; Zarnowski *et al.*, 1999).

Moreover, limited information is available on the effect of genotype and growth conditions on the concentration and composition of AR in durum wheat. In a recent paper (Bellato *et al.*, 2013) the authors have shown data on the phytochemical profile of the durum wheat grains also, considering the AR variability. In the present study additional information on the effects of genotype (G), environmental factors (E) and their interaction (G×E) on AR accumulation was provided. As regard the potential of these compounds to inhibit fungal growth, in this work the antifungal activity of 5-(*n*)-alkylresorcinol extract, from durum wheat whole grain, against four species of *Fusarium* (*F. graminearum* Schwabe, *F. culmorum* (W.G.Smith) Sacc, *F. avenaceum* (Fr.) Sacc. and *F. poae* (Peck) Wollenw.) was described.

II – Material and methods

Fifteen Italian commercial varieties of durum wheat (*T. turgidum*, L. ssp. *durum*) were grown in two different geographical areas, Jesi and Foggia located in central-north and southern Italy, respectively, in two successive years (2008–09 and 2009–10) with two replications. The environmental conditions for the selected growing areas were reported previously (Ciccoritti *et al.*, 2011). Moreover, during 2009–10, the Montelibretti area was also included. From sowing time to harvest, temperature and rainfall data were considered normal for this area of central Italy. The samples, immediately after harvest, were milled using a laboratory cyclone mill (Cyclotec 1093, Foss, Italy) to pass a 0.5 mm screen, to produce wholemeal flour.

To maximize the extraction, 1g of milled samples of each variety was extracted with 40 ml of acetone for 24h (Ross *et al.* 2001) and total AR content and relative homologue composition in

the extracts were determined by GC-MS analysis according to Landberg *et al.* (2009). All the analytical details are provided in Ciccioritti *et al.* (2013).

To test the antifungal activity by minimizing co-extraction of interfering substances, AR extract was prepared from durum wheat whole grains by using cyclohexane solvent (ratio 1:5 w:v) (Nocente *et al.*, 2012). The extract was then filtered through Whatman paper, dried and finally redissolved in 1 ml of the solvent.

Then the fungistatic activity against *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* was tested. One 4-mm diameter plug of the isolates growing on PDA was placed in the center of the 60 mm PDA plates previously prepared by spreading 500 µl of the extract. After 4 to 6 da from inoculation the diameter of colonies was measured and compared to control plates and the percentage of growth inhibition was calculated.

The combinations of years and locations were treated as five environments. Analysis of variance was performed with the MSTATC program (Michigan State University, East Lansing, MI) using a factorial model (mod.9) with G, E (locality and year) and G x E interaction. Genotype means were separated using Duncan's multiple range test ($p < 0.05$) by combining the results across environments. Principal component analysis (PCA), performed with MATLAB software (R2010a version, MathWorks Inc., USA), was used to study the variation associated with the genotype and the environment.

III – Results

1. Variability of AR in relation to genotype and environment factors

On average, the analysed durum wheat genotypes showed a variability range from 173.9 to 415.2 µg/g (dry matter, DM). Data for the 15 durum wheat common cultivars grown in the selected five environments were analyzed by ANOVA (Table 1).

Table 1. Mean value, standard deviation (SD) and mean squares of genotype (G), environment (E) and interaction (G x E) for 5-n-alkylresorcinol (AR)

Source of variation	Degrees of freedom	Mean square
Replication	1	172.83
Genotype (G)	14	6194.93***
Error	14	205.5
Environment (E)	4	24680.30***
G x E	56	2418.08***
Error	74	299.31
<i>P</i> <0.001*** Mean value (%DM) ± SD=		264.0 ± 32.9

Results showed that both G and E had highly significant effects on AR content ($p < 0.001$). In addition, the contribution of G x E interaction to the total variability was lower than that of the genotype or environment alone. Anderson *et al.* (2010) also reported a strong influence of genotype as well as of environment on the AR content in bread wheat. Our data showed that E was the main factor contributing to the total variation in the parameters that were measured.

Box plots were used to compare the variation in the AR content within and between environments (Fig. 1).

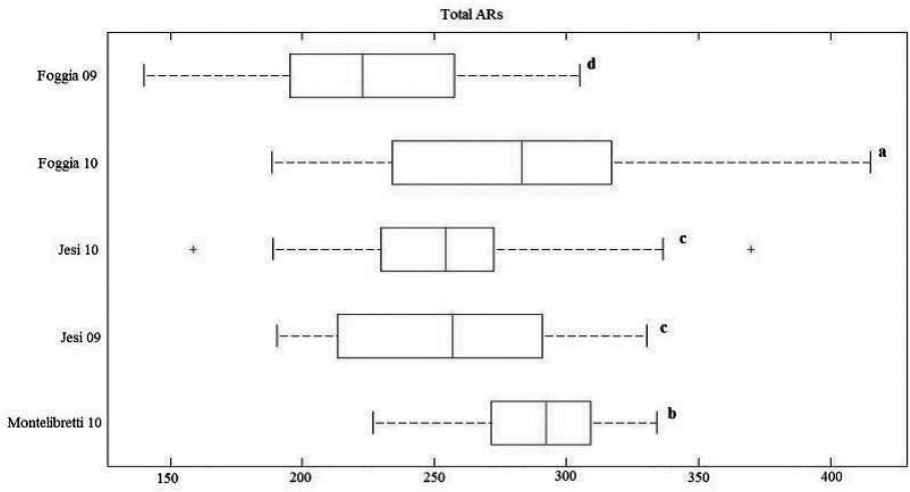


Figure 1. Dataset box plots: evaluation of E variability on AR content ($\mu\text{g/g DM}$). Different letters indicate that means are significantly different from each other ($P < 0.05$).

The AR content showed a significantly higher mean value in Foggia 2010 ($302.7 \mu\text{g/g DM}$) with a range between 198.0 and $415.2 \mu\text{g/g DM}$. On average, the lowest AR content ($241.7 \mu\text{g/g DM}$) was recorded in Foggia in 2009 and the highest in Foggia in 2010, which is the environment where the lowest amount of precipitation occurred. These results are in agreement with Anderson and co-workers (2010) who have observed a great variability in the AR content of bread wheat between years and locations, with highest contents in an environment characterized by hot dry conditions during grain filling.

Box plots of the 15 common varieties grown in all of the environments, as well as the variation in the measurement within a single variety are shown in Fig. 2.

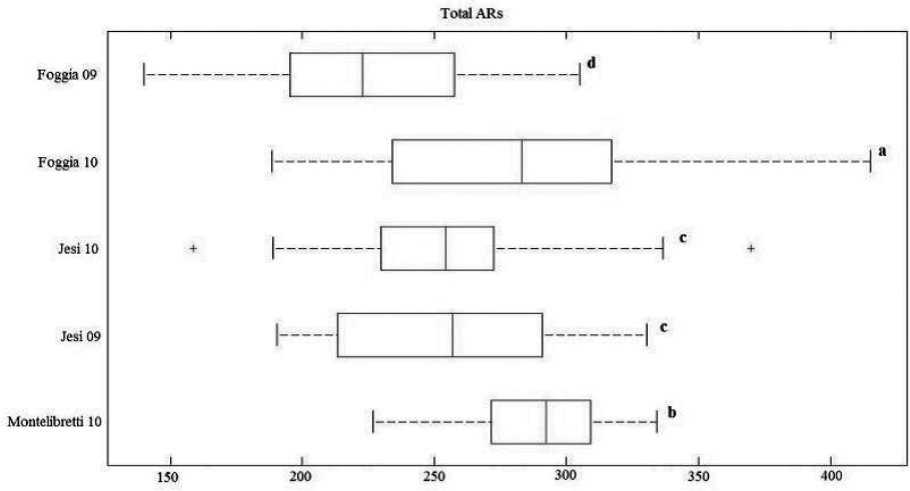


Figure 2. Dataset box plots: evaluation of G variability on AR content ($\mu\text{g/g DM}$). Different letters indicate that means are significantly different from each other ($P < 0.05$).

The genotypes significantly differed in their AR content and the mean values ranged from 210.0 (Normanno) to 324.1 $\mu\text{g/g DM}$ (Iride). The box plots show limited variation in some of the genotypes (Minosse, Normanno, Tirez, Ciccio, and Simeto) underlying the significant contribution of genotypic characteristics to the AR accumulation. The characterization of AR extracts by using GC-MS technique showed no significant differences of homologue composition in the analyzed durum wheat cultivars, which presented high proportion of homologues with high chain length C21:0, C23:0 and C25:0. This is in agreement with previous findings that the variations in AR composition are mainly due to *Triticum* species (Ciccoritti *et al.*, 2013). The effect of the environment on AR content can be clearly seen by PCA of the varieties and environments (Fig. 3). The environments are in similar positions on the positive side of PC1 (explained variance = 66%); in the biplot Foggia 2010 and Montelibretti 2010 (the latter appears very close to the axes) are located on the positive side for PC2 (explained variance = 30%). The varieties with positive scores for PC1 and PC2 generally had AR content slightly above the average. Among them, only Iride (positive value for PC1) showed higher and more stable contents of AR across environments. Four varieties with positive scores for PC1 showed very strong interaction with the environment and had the highest contents of AR in Foggia 2010.

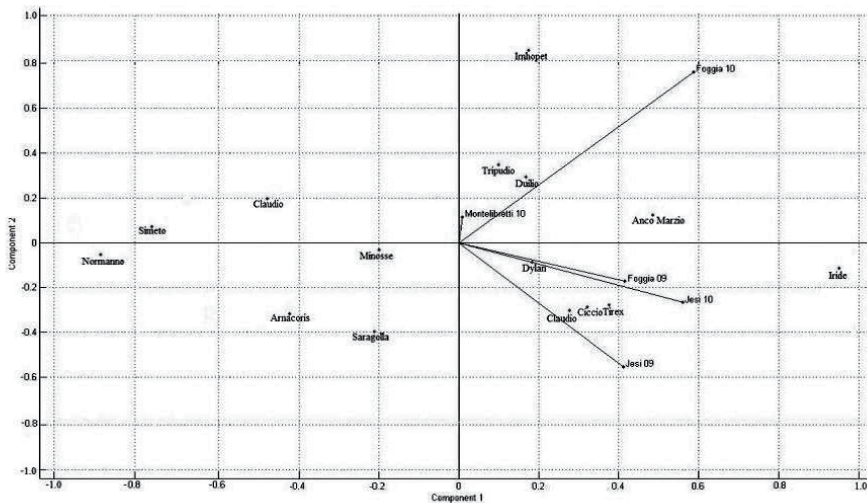


Figure 3. Genotype \times Environment biplot from PCA for AR content of 15 durum wheat varieties grown in five environments.

2. Antifungal activity of ARs

Following the indications of previous study (Nocente *et al.*, 2012), to test the antifungal activity of AR extract from durum wheat grain, cyclohexane solvent was used. Gas chromatography (GC-MS) analysis confirmed that cyclohexane AR extract contained a higher proportion of C21:0 homologue, which represented 59.9% of ARs, and lower percentages of the other homologues. Moreover in agreement with Zarnosky *et al.* (2004) the GC-MS analysis showed low amount of ballast substances co-extracted with AR (*data not shown*).

The fungistatic activity of cyclohexane extract on mycelial growth of *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* was tested (Fig.5).

cyclohexane was chosen as extractant of 5-n-alkylresorcinols from intact kernels. In these analytical conditions, our data demonstrated antifungal activity of AR extracts from durum wheat grain against the tested *Fusarium* fungi; in particular, high growth-inhibiting activity of the extract was evidenced against *F. avenaceum*, this fungus appeared more sensitive towards AR than *F.graminearum*, *F. culmorum* and *F. poae*. The results suggest that the ARs can be considered the major components responsible for the fungistatic properties of cyclohexane extract from durum wheat grain. However, it cannot be excluded that the minor components in the extract may also contribute to the antifungal activity.

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