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Variability of total antioxidant capacity among durum wheat genotypes

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Abstract. Durum wheat (*Triticum turgidum* L., ssp. *durum)* contains many health-promoting components involved in different biological activity, partly attr buted to potential chemo-preventive substances (phytochemicals), including antioxidants present in high amounts in vegetable crops and also in cereal grains. Recently, the determination of total antioxidant capacity (TAC) has gained a growing interest as a tool for exploring the putative role of antioxidant-rich products in the prevention of degenerative diseases and for the selection of varieties with potentially positive health benefits. The aim of the present study was to determine the influence of the genotype, year and environment on the TAC level in different cultivars of durum wheat during 2009, 2010 and 2011 crop years and in 3 environments. The results showed that year (Y), environment (E) and genotype (G), as well as their interactions, significantly influenced the TAC value in the durum wheat grains. Principal component analysis (PCA) identified genotypes with high and stable TAC values over the environments. Correspondence analysis and boxplots were also useful for assessing more stable cultivars over the years. Among different genotypes, the TAC values ranged between 36,55 to 55,83 mmolTEAC/kg (dry matter, DM).

Keywords. Durum wheat – TAC – Genotype – Environment.

Variabilité de la capacité antioxydante totale parmi les génotypes de blé dur

Résumé. *Le blé dur (Triticum turgidum L., ssp. durum) contient de nombreux composants bénéfiques pour la santé impliqués dans différentes activités biologiques, en partie attribués à des substances chimio-préventives potentielles (phytochimiques), incluant les antioxydants présents en grande quantité dans les cultures maraîchères et aussi dans les grains de céréales. Récemment, la détermination de la capacité antioxydante totale (CAT) a suscité un grand intérêt comme outil pour explorer le rôle potentiel des produits riches en antioxydants dans la prévention des maladies dégénératives et pour la sélection de variétés avec des qualités potentiellement bénéfiques pour la santé. Le but de cette étude était de déterminer l'influence du génotype, de l'année et de l'environnement sur le niveau de la CAT dans différents cultivars de blé dur durant les années de culture 2009, 2010 et 2011 et dans 3 environnements. Les résultats ont montré que l'année (Y), l'environnement (E) et le génotype (G), ainsi que leurs interactions, ont influencé de façon significative la valeur de la CAT dans les grains de blé dur. L'analyse en composantes principales (ACP) a identifié des génotypes avec des valeurs élevées et stables de CAT pour les environnements. L'analyse des correspondances et des boxplots a également été utile pour évaluer les cultivars les plus stables au fil des années. Parmi les différents génotypes, les valeurs de la CAT ont varié entre 36,55 à 55,83 mmolTEAC / kg (matière sèche, MS).*

Mots-clés. *Blé dur – CAT – Génotype – Environnement.*

I – Introduction

Epidemiological studies underlined the potential protective role of consumption of whole-grain cereals against several chronic diseases (Thompson 1994). These health benefits have been partly attributed to a wide variety of potential chemo-preventive substances, so-called phytochemicals, including antioxidant compounds present in high amounts in different vegetable crops and also in cereal grains (Frusciante *et al.* 2007, Serafini *et al.* 2002). The global action of all antioxidant compounds present in a raw material is generally expressed as total antioxidant

capacity (TAC). This parameter has gained a growing interest as a tool for exploring the putative role of antioxidant-rich products in the prevention of degenerative diseases, as well as for the selection of varieties/species rich in bioactive compounds with potentially positive health benefits. Wojdylo and Oszmainski (2007) measured in oat the free radical scavenging ability of methanolic and enzymatic extracts comparing the DPPH radical method (Yen and Chen, 1995) and the ABTS radical method (Re *et al.* 1999); other authors (Lavelli *et al.* 2009, Hidalgo *et al.* 2006) measured the total free radical scavenging capacity with DPPH radical of extracts from different wheat species using different extraction protocols and solvents. Several methods have been developed to measure the whole matrix antioxidant capacity and data are often variable and underestimated due to the different extraction methods (Frankel *et al.* 2008), considering that there is not a unique solvent suitable to solubilize all antioxidants present in a complex food matrix and generally the extraction procedures employ hydrophilic or lipophilic solvents and then measure the TAC separately. More recently a procedure for the measurement of the total antioxidant capacity was developed by Serpen *et al* (2008) without the extraction step, but directly on the solid food matrix. This method, was deeply evaluated by Gokmen *et al.* (2009) and defined as QUENCER (Quick, Easy, New, CHEap and Reproducible); it overcomes the difficulties of the extraction step, highlighting the synergistic effect of different antioxidants molecules, partially lost during antioxidants extraction or during the measurements on different extracts.

The aim of the present study, carried out in the AGER project “From seed to pasta” was to determine in a group of durum wheat cultivars the influence of genotype, year and environment on the total antioxidant capacity measured by a direct method.

II – Material and methods

Twenty durum wheat cultivars (Achille, Alemanno, Anco Marzio, Arnacoris, Biensur, Ciccio, Claudio, Creso, Duilio, Dylan, Iride, Minosse, Neolatino, Latinur, Liberdur, Severo, Saragolla, Simeto, Tirex, and Trionfo) were grown in Montelibretti (Rome) during 2009, 2010 and 2011 in a national network experimental trial; a set of 10 cultivars, Anco Marzio, Ciccio, Claudio, Creso, Duilio, Dylan, Iride, Latinur, Saragolla and Simeto, grown during the same period in other two locations (Jesi, Foggia) representing the different agroclimatic areas typical of durum wheat crop in Italy, were considered for evaluating the environment influence. Grain samples were ground with a laboratory mill and a sieving of 1 mm (Cyclotec, PBI), to obtain wholemeal employed for the TAC determination, applying the TEAC direct method described by Serpen *et al.* (2008). The TAC analytical procedure is based on an immersion of a pulverized solid matrix (sample) in a 50% ethanol solution containing 2,2’-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical. The final radical absorbance was set at 0.7 nm and the solution with the solid sample was incubated in an orbital shaker (190 rpm) at 25°C for 50 min. The absorbance measurements were performed at 734 nm. The antioxidant capacity was expressed as mmol of Trolox equivalent antioxidant capacity (TEAC) per kg of *2* sample by means of a Trolox dose-response curve. All samples were diluted with cellulose powder (1:9 w/w), inert toward the ABTS reagent, to obtain an absorbance in the valid range of the calibration curve. The TAC analysis was performed in triplicate.

The effects of year (Y), genotype (G) and environmental factors (E) and their interactions on TAC values have been studied using different statistical approaches. Analysis of variance (ANOVA) was performed by MSTATC program (Michigan State University, East Lansing, MI). Principal component analysis (PCA) and correspondence analysis were performed by MATLAB software (R2010a version, MathWorks Inc., USA) to study variation associated with genotype and environment for TAC values.

III – Results

1. Genotype influence

Mean TAC value for 20 cultivars grown in Montelibretti during three years was 43.64±3.03; the highest value was performed by Trionfo in 2009 (49.64 mmol TEAC/kg) and the lowest by Alemanno in 2010 (35.78 mmol TEAC/kg). All genotypes showed a different year-dependent behaviour (Fig. 1). The data show that genotypes influenced the TAC level, but a year-genotype interaction is always present, as also pointed out by ANOVA (Table 1).

Table 1. Analysis of variance: mean square and F value of year, environment and their interaction.

Sources	DF	Mean squares	F value
Replication (R)	2	3.103	1.54
Year (Y)	2	3.972	1.97
RxY	4	2.020	
Genotype (G)	19	35.340*	15.68
YxG	38	22.344*	9.92
Error	114	2.253	

**Significant at P<0.001.*

Figure 1. TAC of different *T. durum* cvs grown in Montelibretti during three crop years.

The whole data set was converted to a frequency table considering the cultivar as first variable and the TAC value as second variable, identifying for this last variable three arbitrary categories (low, medium, high) based on the mean values and standard deviations. Cultivars were graphically located near Low, Medium and High TAC values (Fig. 2). In the graphical representations of the frequency table the distances between the points representing the cultivars are a measure of the similarity of the cultivars-frequency profiles. Each trait-category point will lie close to the cultivars

for which the trait category is prominent. In particular, in terms of frequencies the cultivars Trionfo and Iride are the highest while Achille and Alemanno are the lowest (Fig. 2).

2. Environment and year influence

The behaviour of 10 cultivars during 3 years was quite different in 3 environments, with the exception of Montelibretti where TAC level was rather stable. Among the cultivars (Fig. 3), Ciccio and Duilio showed high TAC variability in the environments during the 3 years, while Iride was quite stable. The combined ANOVA on 10 cultivars grown in 3 environments during 3 years (Table 2) showed that TAC is mostly influenced by year (Y), followed by environment (E) and then by genotype (G), the interaction YxE was also significant.

Factor analysis (PCA) used to evaluate simultaneously all variables and their relationships (Fig. 4) identified that two factors explaining 80% of the total variance: The first factor (PC1) appears mainly linked to TAC and explained the 48.85% of the total variance, while the second (PC2) seems mainly associated to the cultivar stability and explained the 30.21% of the total variance. All environments were in similar positions on the positive side of PC. Jesi 2009 and 2010 and Montelibretti 2010 are located on the negative side for PC2, probably due to different agroclimatic conditions (i.e., high seasonal rainfall). Among the cultivars, Simeto had the highest TAC values, but was not stable across the environments, Iride presented a high TAC value and high stability; Creso and Claudio had lowest TAC values and seem less affected by environment (Fig. 4).

Figure 2. Correspondence analysis of the twenty cvs grown in Montelibretti (RM) in the three years.

Figure 3. Boxplots of the cultivars behaviour in three environments and during 3 years.

Table 2. Analysis of variance of data from 10 cvs grown at three environments for three years.

Sources	DF	Mean squares	F value
Year (Y)	2	445,75*	111,24
Environment (E)	2	211,25*	52,72
Genotype (G)	9	62,54*	15,61
YxE	4	92,56*	23,10
YxG	18	22,29*	5,56
ExG	18	19,69*	4,91
YxExG	36	17,24*	4,30
Error	180	4,01	

**P significant P<0.001*

Figure 4. PCA analysis of the durum cvs grown in three environments and three years: year and environment effects.

IV – Conclusions

In this study we were able to classify some durum cultivars on the basis of total antioxidant capacity (TAC). It was possible to evaluate the influence of year, environment and genotype on the TAC, highlighting the year as the main factor affecting the antioxidant capacity followed by environment and genotype; moreover it was possible to evaluate the cultivar stability across years. The results suggested that it is possible, on the basis of TAC values, to choose the more suitable cultivars for use in breeding programs to select varieties naturally rich in antioxidants.

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