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Phenolic acids profile, nutritional and phytochemical compounds, antioxidant properties in colored barley grown in southern Italy

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ABSTRACT

Free, soluble conjugated, and insoluble bound phenolic acids and some of the main antioxidant phytochemicals (i.e., total polyphenols, proanthocyanidins, carotenoids) were investigated in 20 genotypes of colored barley. These included 16F8 recombinant inbred lines (RILs) obtained from crosses of four parental lines: '2005 FG', 'K4-31, 'L94', and 'Priora'. The aim of this work was these to promote the introduction of new barley genotypes with high contents of such natural antioxidants, and of dietary fiber. These new genotypes will enlarge the market of novel functional foods. Large variations were seen in the contents of phytochemicals and β-glucans across these barley genotypes. The highest protein (14.4%) and β-glucan (4.6%) contents were in the blue naked parental genotype '2005 FG'. Overall, insoluble bound phenolic acids represented 88.3% of the total phenolic acids, and ferulic acid was the main conjugated phenolic acid. Salicylic and gallic acids were the most represented among the free phenolic acids, with no *p*-coumaric and cinnamic acids detected. Total polyphenols and proanthocyanidins were highest in the RILs '3009′ (2917 μg g^{-1}) and '1997′ (1630 μg g^{-1}). The barley line with high total polyphenols (RIL '3009′) also showed the highest antioxidant capacities (by both DPPH and ABTS methods: 13.4 μmol g^{-1} and 15.6 μmol g^{-1}, respectively). Among the RILs examined, '3004′, '3008′, and '3009′ showed 30% higher antioxidant capacities than their parentals, thus providing potential health-promoting benefits.

1. Introduction

Barley (*Hordeum vulgare* L.) is an important crop for direct human consumption, animal feeds, and industrial applications. It is also a highly adaptable cereal crop that is cultivated across a wide range of climates around the world. Barley is therefore readily available and relatively inexpensive.

Barley grain is mainly used as a source of carbohydrate, but it also contains protein, fat, crude fiber, mineral matter, calcium, and phosphorus (Baik & Ullrich, 2008). Barley grown for human consumption is commonly used in breads and soups, and as a source of malt for alcoholic beverages, and especially for beer (Izydorczyk & Dexter, 2008). However, recently, barley has gained renewed interest as an ingredient in the production of healthy food products, due to the functional properties of its bioactive compounds, which include tocols (e.g., vitamin E) and β-glucans (i.e., soluble fiber) (Bonoli, Verardo, Marconi, & Caboni, 2004). β-Glucans reduce the risks associated with cardiovascular disease by lowering serum cholesterol, and they are also beneficial to diabetics through regulation of blood glucose levels (Braaten et al., 1994). Many people are becoming aware that because of the presence of these bioactive compounds or chemicals, certain foods can have positive impacts on the health, physical well being, and mental state of humans.

Some barley cultivars have colored kernels because they contain anthocyanins, carotenoids, phenolic acids, flavonols, flavans and/or other phytochemical compounds that can be synthesized in plants through secondary metabolism. These phenolic compounds are considered to be potent antioxidants, free radical scavengers, and metal chelators, and to inhibit lipid peroxidation (Cook & Samman, 1996; Fattore et al., 2016; Nascimento et al., 2018; Rocchetti et al., 2017; Shen et al., 2016; Shen, Li, Jing, & Zheng, 2018). Epidemiological studies and associated meta-analyses have strongly suggested that long-term consumption of diets rich in plant polyphenols can offer protection against development of cancers, cardiovascular disease, brain and immune dysfunction, diabetes, osteoporosis, and neurodegenerative diseases (Pandey & Rizvi, 2009; Rondini, Peyra-Maillard, Marsser-Baglieri, & Berset, 2002; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

The flavans represent 58% to 68% of the total phenolic content of

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barley, and the most abundant flavanols are formed by the proanthocyanidins, in their dimeric (e.g., prodelphinidin B3, procyanidin B3) and trimeric (e.g., procyanidin C2, prodelphinidin C2, other prodelphinidin isomers) forms (Dvořáková, Moreira, Dostalek, Skulilova, Guido, and Barros, 2008; Goupy, Hugues, Boivin, & Amiot, 1999; Kim et al., 2007). The phenolic acids in barley grain belong to the two groups of benzoic acids and cinnamic acids, and their derivatives, and they can be both free and bound (Bonoli et al., 2004). In contrast to free phenolic acids, bound phenolic acids are ester-linked to the cell walls and require acid, base or enzymatic hydrolysis for their release from the cell matrix (Pandey & Rizvi, 2009).

It has been reported that the major portion of phenolics in grain are in the insoluble bound form (corn, 85%; oats and wheat, 75%; rice, 62%), and these compounds are the major contributors to total antioxidant activity (wheat, 90%; corn, 87%; rice, 71%; oats, 58%) (Adom & Liu, 2002). These bound phytochemical compounds have been often ignored, and thus the contents of phenolic compounds was underestimated. Phenolic acids scavenge free radicals that are responsible for promoting lipid oxidation at biologically significant rates (Decker, 1998). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid) are the major low-molecular-weight phenolic acids in barley grain, and although mainly in the outer layers (i.e., husk, pericarp, testa, aleurone), they have also been detected in the endosperm. The other bound phenolic acids in barley include vanillic, sinapinic, and *p*-hydroxybenzoic acids (Dvořáková, Guido, Dostálek, Skulilová, Moreira, and Barros, 2008; Goupy et al., 1999; Kim et al., 2007).

Several methods have been proposed to measure antioxidant activities of vegetable and plant extracts, although discrepancies in the data obtained have been reported. These differences appear to be due to the contents of the phenolic compounds, the environmental conditions and the procedures. The methods mainly used are based on 1,1- diphenyl-2-picrylhydrazyl organic radical (DPPH), Trolox equivalent antioxidant capacity (TEAC), and 2–2-O-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) organic cation radicals. In particular, the ABTS method is often used to determine antioxidant potentials of pigmented compounds and extracts, as interference in this assay can be avoided (Prior, Wu, & Schaich, 2005).

A naked barley breeding program was started with the purpose of introducing the hulless gene into the breeding material and to produce varieties that are rich in antioxidant compounds that can be specifically adapted for food use. In the present study a set of advanced breeding lines of barley selected for the colored outer layers of the naked kernel were compared, with the aims to identify and quantify their major phenolic compounds, and to investigate correlations between their phytochemicals constituents and their antioxidant capacities.

2. Materials and methods

2.1. Plant materials

A set of 16 F8 recombinant inbred lines (RILs) were obtained by single-seed descent, and were grown with their respective parents in the experimental fields of the Cereal Research Centre (CREA-CI) in Foggia (southern Italy) (41°28′N, 15°34′E; 76 m a.s.l.) during the 2015–2016 growing season. The RILs were screened for colored and naked seeds, and were developed through three different parental backcross programs (Table 1). These parentals are defined as group A, and were: (i) line '2005 FG', with blue and naked seeds, selected from a population of malting barley; (ii) RIL 'K4–31', with black and hulled seeds, as derived from an 'L94' × 'Vada' cross; (iii) line 'L94', with black and naked seeds; and (iv) cv. 'Priora', with white and naked seeds and rich in ß-glucans, as derived from an 'Arda' × 'Mondo' cross. The cross of 'K4–31' × 'Priora' × '2005 FG' also defined group B with seven RILs included ('1956', '1960', '1966', '1981', '1997', '2000', '2002'), and the cross of 'Priora' × 'K4–31' × '2005 FG' defined group C with nine RILs included ('3001', '3002', '3003', '3004', '3005', '3006', '3007', '3008', '3009') (Table 1).

Table 1
Barley samples (cultivar or breeding lines) investigated in this study and their characteristics.

	Sample	Characteristic	
		Pigmentation	Hulled/ Naked
Parental lines (A - Group)	2005 FG	Blue	Naked
	K4–31	Black	Hulled
	L94	Black	Naked
	Priora	White	Naked
K4–31 × Priora × 2005FG (B - Group)	1956	Blue	Naked
	1960	Blue	Naked
	1966	Blue	Naked
	1981	Blue	Naked
	1997	Blue	Naked
	2000	Blue	Naked
	2002	Blue	Naked
Priora × K4–31 × 2005FG (C - Group)	3001	Blue	Naked
	3002	Blue	Naked
	3003	Blue	Naked
	3004	Blue	Naked
	3005	Blue	Naked
	3006	Blue	Naked
	3007	Blue	Naked
	3008	Blue	Naked
	3009	Blue	Naked

The trial was performed on a clay-loam soil (Typic Chromoxerert) with the following characteristics: 36.9% clay, 50.5% silt, 12.5% sand, pH 8 (in H_2O), 15 mg kg^{-1} available phosphorus (Olsen method), 800 mg kg^{-1} exchangeable potassium (NH_4Ac), 1.5 g kg^{-1} total nitrogen, and 21 g kg^{-1} organic matter (Walkey–Black method). The land was ploughed, hoed and harrowed twice prior to planting.

Sowing was performed with a plot driller on December 10, 2015, at a seeding density of 350 viable seeds m^{-2}. Weed control was carried out at the end of tillering, using Manta Gold (fluroxipir, 6.0% [60 g L^{-1}]; clopiralid, 2.3% [23.3 g L^{-1}]; [4-chloro-2-methylphenoxy] acetic acid, 26.7% [266 g L^{-1}]) mixed with Axial Pronto (pinoxaden, 6.4% [60 g L^{-1}]; cloquintocet-mexyl, 1.55% [15 g L^{-1}]). The fertiliser used at sowing was 18/46 fertiliser (18% elemental nitrogen; 46% P_2O_5; by weight) applied at 200 kg ha^{-1}, and at plant tillering was NH_4NO_3 (26% elemental nitrogen) applied at 200 kg ha^{-1}.

A randomised complete block design with three replications was used. The plot area was 10 m^2, with 8 rows that were each 7.5 m long and 0.17 m apart. The plants were harvested mechanically after physiological maturity (June 14, 2016), as the six central rows, using a plot combine harvester. The grain were stored in a cool chamber at 5 °C until analysis. Before analysis, the barley samples were ground using a sample mill (Cyclone; Udy Corp., Fort Collin, USA) equipped with a 0.5-mm screen. The chemical compositions were analysed as two subsamples of each grain sample.

2.2. Chemicals and reagents

The solvents methanol, acetonitrile, ethyl acetate, acetone, diethyl ether, and phosphoric acid were from Carlo Erba (Milan, Italy) and were HPLC grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetra-methyl-chroman-carboxylic acid (Trolox), 2,2–0-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), the cathechin standards and Folin–Ciocalteu's phenolic reagent were from Sigma–Aldrich Co.(Milan, Italy). All of the solutions used were made with deionized water (Milli-Q; Millipore, Bedford, USA). The phenolic compound standards benzoic acid, caffeic acid, chlorogenic acid, *trans*-cinnamic acid, *m*-coumaric acid, *p*-coumaric acid, *o*-coumaric acid, 3,4-dimethoxy-benzoic acid, ferulic acid, gallic acid,