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Effect of sowing time and soil water content on grain yield and phenolic profile of four buckwheat (*Fagopyrum esculentum* Moench.) varieties in a Mediterranean environment☆

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ABSTRACT

Four varieties of buckwheat (*Fagopyrum esculentum* Moench), Aizu, Botan, Hitaci and Kitawase, were evaluated using two sowing times and two irrigation treatments in a Mediterranean environment in order to ascertain a possible adaptation of this crop to this environment. Buckwheat is cultivated in particular for the high protein content and for its good biological value compared to common cereals, and for its high levels of phenolic compounds. In addition, it is gluten-free and thus suitable for people suffering from celiac disease. Overall the 4 buckwheat varieties tested in this study showed high protein content (14%, on average), although grain yield was low (420 kg ha^{-1}, on average). Yield was affected by sowing time and irrigation treatment, whereas protein content was related to the variety. Low irrigation treatment ("water stress") resulted in a lower yield (325.9 kg ha^{-1}) and lower protein content (13.7%) compared to full irrigation treatment (514.7 kg ha^{-1} and 14.3%, respectively), but also in a higher content of phenolic compounds (4.46 *vs.* 2.79 mg/g). Grain yield and phenolic fingerprint of buckwheat seeds were investigated in order to establish differences and similarities related to variety, sowing time and soil water content.

1. Introduction

Buckwheat is a summer-growing crop belonging to the Polygonaceae family, cultivated mainly in Russia, China, Kazakhstan and Ukraine, but it can be found elsewhere, especially in Asia, Europe, Canada and the USA (FAOSTAT, 2013; Giménez-Bastida and Zieliński, 2015). Among the several buckwheat species, only two are cultivated for human consumption: common buckwheat (*Fagopyrum esculentum* Moench), which represents up to 90% of total buckwheat production, and tartary buckwheat (*Fagopyrum tataricum* Gaertner), with a smaller and more bitter seed (Campbell, 1997; Zhao et al., 2012). Although buckwheat does not belong to the graminaceous family, it is considered a pseudo-cereal because flour for food products is produced from its grains, as with the cereals (Christa and Soral-Smietana, 2008).

Buckwheat can be grown under a variety of climatic conditions in a wide range of soils. It completes its crop cycle rapidly, reaching maturity within 3–4 months or less (Tsuneo, 2004; Arduini et al., 2016). Cultivating this crop has a very low impact on the environment since it does not require the use of pesticides, and it thrives even with low doses of fertilizer and reduced volumes of water (Campbell, 1997). For these reasons, buckwheat is considered an alternative, easy-to-grow, short-season grain crop. It produces characteristic dark-hulled, three-angled, starch-filled achenes commonly called seeds. Growth is indeterminate, that is, the seeds do not all mature at the same time (Halbrecq et al., 2005).

Buckwheat has high protein content with a well-balanced amino-acid composition (Mota et al., 2016). It can be eaten by people suffering from celiac disease because it contains no gluten (Ikeda, 2002; Torbica et al., 2012). Buckwheat is used mainly as an ingredient for bread, rice, soup, cakes, noodles, cookies and so on. In recent years because buckwheat has been perceived as a health-promoting grain, it has become a highly appreciated ingredient in the preparation of so-called functional foods (Li and Zhang, 2001; Giménez-Bastida and Zieliński, 2015; Gao et al., 2016). It is well known that buckwheat contains a large quantity of phenolic compounds (Sakač et al., 2011; Kiprovski et al., 2015). In particular, it is an important source of rutin (quercetin 3-*O*-rutinoside) (Inglett et al., 2011; Verardo et al., 2010; Kiprovski et al., 2015). Rutin is a flavonoid with known antioxidant, anti-

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inflammatory, anti-carcinogenic and anti-hemorrhagic effects, as well as cardiovascular benefits and blood-vessel protecting properties (Van Acker et al., 1996; Baumgertel et al., 2003; Bai et al., 2015).

Kalinova and Vrchotova (2011) and Kiprovski et al. (2015) reported that variations in antioxidant activity and polyphenol composition in buckwheat could depend upon variety, environmental conditions and crop management. Because they are secondary metabolites, the presence and content of polyphenols in plants depends both on genetics and external factors; the analysis of variations in levels and composition of phenolic compounds, supported by adequate statistical tools, is currently considered to be a powerful method of monitoring plant system response (Siracusa and Ruberto, 2014).

Although buckwheat in the Mediterranean environment is cultivated only in small areas, it is currently receiving renewed attention from the research community and consumers. However, in spite of its many qualities, the appropriate varieties and sowing times for buckwheat in the Mediterranean environment have not yet been clearly identified. With this in mind, grain yield and the phenolic fingerprint of buckwheat seeds were studied looking for differences and similarities in variety, sowing time and soil water content.

2. Materials and methods

2.1. Field experiment

The trial was carried out in 2008 and 2009 in an experimental field located in southern Sicily, Italy (Catania, 10 m a.s.l., lat 37° 31′ 00″N, long 15° 4′00″E) on a clay soil, well-endowed with the main mineral elements (N 1.53‰, P 23.08 ppm and K 364.91 ppm). The following factors were studied: 4 different cultivars (Aizu, Botan, Hitaci, Kitawase), 2 sowing times (September 2008 and May 2009) and 2 irrigation treatments (fully irrigated, with each irrigation at 80% of field capacity; and water stressed, which is a supplementary-type of irrigation with only three interventions at critical moments). A randomized block design was adopted with plots of 12 m^2 (3 × 4 m) with three replications. Sowing was arranged with a density of 130 plants m^{-2} (15 cm between rows, 5 cm between plants). Weeds were controlled manually. At sowing, 80 kg ha^{-1} of urea equivalent to 36.8 kg ha^{-1} of N and 200 kg ha^{-1} of phosphate equivalent to 40 kg ha^{-1} of P_2O_5 were distributed. After sowing all plots were irrigated to allow seed germination. Water was supplied with a drip system: in the full irrigation treatment water was restored to 100% of maximum evapotranspiration, and supplied each time 80% of field capacity was reached; in the water-stress treatment, water was supplied at three crop stages only: sowing, vegetative stage and flowering, which represented considerable savings in water resources. The crop water use was calculated by class A pan evaporation, using half of the soybean coefficient. At the end of the trial, on an area of 2 × 3 m in the middle of each plot to avoid any edge effect, the following were determined: plant height, 1000 seed weight and grain yield.

Temperature and rainfall were recorded throughout the entire experiment by a meteorological station (Data logger CR10 Campbell Scientific, Inc., Logan, UT, USA) located next to the experimental field. In 2008, during the trial period of the first sowing time (September–February), the average temperature ranged from 24.5 °C at the beginning of September to about 9 °C during the coldest period (December–February); the highest and lowest temperatures were 30.7 °C and 1.7 °C, respectively, in September and February. Rainfall was approximately 495 mm and almost all of it fell during the first 4 months. During the second sowing time (May–August), temperatures ranged from 16.9 to 25.7 °C with a peak of 32.2 °C in June and the lowest temperature of 11.8 °C in May. Rainfall (13 mm) occurred in May. Temperatures and rainfall during the trial were similar to data from the previous 30 years (see online Supplementary Fig. S1).

2.2. Chemicals

All solvents and reagents used in this study were high-purity laboratory solvents from Carlo Erba (Milano, Italy). HPLC grade water and acetonitrile were obtained from VWR (Milano, Italy). Pure vitexin (apigenin 8-*C*-glucoside), orientin (luteolin 8-*C*-glucoside) and quercetin 3-*O*-glucoside were provided by Extrasynthese (Lyon, France); rutin (quercetin 3-*O*-rutinoside), catechin and quercitrin (quercetin 3-*O*-rhamnoside) were obtained from Fluka (Sigma-Aldrich s.r.l., Milano, Italy), while kaempferol 3-*O*-rutinoside and caffeic acid were provided by Sigma (Sigma-Aldrich s.r.l., Milano, Italy).

2.3. Protein content

Seeds of each plot were ground in a blade grinder ensuring that the temperature never exceeded 30 °C. Grain protein content was determined by Kjeldahl method (N × 6.25) (AOAC, 2000).

2.4. Chromatographic analyses

2.4.1. Sample preparation

Extraction of buckwheat seeds for the analysis of polyphenols was performed as follows: 250 mg of the finely ground seeds were put into 8 mL sample vials, to which 3 mL of an acidic aqueous methanol solution (80% methanol, 19% water, 1% formic acid) were added. Samples were then maintained at room temperature (20 °C) overnight under vigorous stirring. The resulting heterogeneous mixtures were transferred into sample tubes, then centrifuged at 1182*g* for 10 min. The resulting clear yellowish solutions were finally put into 2 mL amber vials and sent for analytical determinations. When required, the analytical samples were stored for short periods (one week at the most) at −20 °C under nitrogen atmosphere.

2.4.2. HPLC/DAD quantitative analyses

Quantitative analyses were carried out on an Ultimate3000 UHPLC focused instrument equipped with a binary high-pressure pump, a photodiode array detector (DAD), a thermostatted column compartment and an automated sample injector (Thermo Fisher Scientific, Inc., Milano, Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. Chromatographic runs were all performed using a reverse-phase column (Gemini C_{18}, 250 × 4.6 mm, 5 µm particle size, Phenomenex Italia s.r.l., Bologna, Italy) equipped with a guard column (Gemini C_{18} 4 × 3.0 mm, 5 µm particle size, Phenomenex Italia s.r.l., Bologna, Italy). Buckwheat polyphenols were eluted with the following gradient of B (1% formic acid in acetonitrile) in A (1% formic acid in water): 0 min: 5% B; 10 min: 15% B; 30 min: 25% B; 35 min: 30% B; 50 min: 90% B; 57 min: 100% B, which was kept for a further 7 min, for a total time of 64 min. This method was used for the first time in this study to analyse this matrix. The solvent flow rate was 1 mL/min. Quantifications were carried out at 350 nm using rutin (R^2 = 0.9999), orientin (R^2 = 0.9997), quercitrin (R^2 = 0.9998), quercetin 3-*O*-glucoside (R^2 = 0.9999) and kaempferol 3-*O*-rutinoside (R^2 = 0.9999) as external standards; the detector was set at 280 nm to build the calibration curve for catechin (R^2 = 0.9997), while vitexin (R^2 = 0.9997) and caffeic acid were quantified at 330 nm using the corresponding reference substances (R^2 = 0.9999 and R^2 = 0.9998, respectively). All analyses were carried out in triplicate.

2.4.3. Identification of main components via HPLC/DAD/ESI/MS

In order to unambiguously identify the chromatographic signals and/or to confirm peak assignments, a series of HPLC/DAD/ESI–MS analyses were performed on a selected number of samples. Variable aliquots (1.0–1.5 mL) of the above-mentioned hydro-alcoholic solutions from quantitative analyses (see Section 2.4.2) were transferred into standard laboratory vials and dried *in vacuo* with a rotary evaporator