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BIOCHEMICAL INVESTIGATION OF TUNISIAN AUTOCHTHONOUS PEARL MILLET (*Pennisetum glaucum* (L.) R. BR) ECOTYPES

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SUMMARY

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is a staple food in many developing countries and constitutes the major source of essential nutrients in semi-arid and arid regions of Africa and India. The knowledge about the nutrient content of autochthonous pearl millet ecotypes is scarce. In this study, biochemical analysis of six Tunisian pearl millet ecotypes has been investigated. Results provide evidence of differences in polyphenols content, antioxidant potential and polyphenol oxidase (PPO) activity among the studied ecotypes. These three parameters were found significantly correlated. Moreover, some ecotypes were shown to be potentially quite important sources of health promoting antioxidants. The studied biochemical properties could be used as criteria of selection for a specific food application especially the integration of this cereal in diverse industrial nutritional products.

Key words: *Pennisetum glaucum,* ecotype, biochemical composition

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is the most drought-tolerant of all the domesticated cereals and is grown in regions where no other cereals can be grown (Chowdari et al., 1998; Sharma and Ortiz, 2000; Maqbool et al., 2001).

It is one of the important crops in semi-arid areas of Africa and India (Andrews and Kumar, 1992; ICRISAT/FAO, 1996). Pearl millet and grain sorghum are staple foods that supply a major proportion of calories and protein to large segments of populations in the semi-arid tropical regions of Africa and Asia (O'Kennedy et al., 2006).

This cereal is also used as excellent forage crop in Australia, southern Africa, South America and the USA because of its low hydrocyanic acid content (Burton et al., 1972; Poncet et al., 2000; Zhan et al., 2003). It has been described as the best summer pasture grass and has great potential to be grown in the U.S. both as a cereal grain and as a pasture grass for ruminant animals (Adeola and Orban, 1995).

In developing countries, the commercial processing of these locally grown grains into value-added food and beverage products is an important driver for economic development (Taylor, 2004). In these countries, there is today a growing demand for gluten-free foods and beverages from people with coeliac disease and other intolerances to wheat who cannot eat products from wheat, barley, or rye (Kasarda, 2001).

Pearl millet is often identified with the poorest of the poor in Africa, where it comprises a significant percentage of the daily food intake. It is known to be nutritionally better than most other cereals (Uprety and Austin, 1972). Its digestibility was 25% higher than for normal sorghum (Ejeta et al., 1988). It's a good source of dietary protein, carbohydrates, fat, vitamins and minerals (Hulse et al., 1980).

Pearl millet is an important source of some minerals particularly iron and zinc (Serna-Saldivar and Rooney, 1995; Adeola and Orban, 1995). It has high levels of lipids, high quality and well-balanced proteins (Elyas, et al., 2002) and diverse health promoting phenolic compounds (Chung and Pomeranz, 1985).

Its health-promoting properties, in particular its antioxidant activity and its use as nutraceuticals and in functional foods are reviewed in the paper by Dykes and Roony (2006). Pearl millet has anti-carcinogenic properties (Van Rensburg, 1981; Chen et al.; 1993).

In Tunisia, pear millet isn't the staple food of rural populations as in the other countries of Africa. Nevertheless, it occupies a very important part of surfaces every year in the centre and in the South of the country (FAO, 2003). All pearl millet production is used for a variety of food products and can potentially be planted as a double crop after winter wheat or barley in southern areas (Radhouane, 2008).

Many autochthonous pearl millet ecotypes have generated interest in Tunisian pearl millet because their nutritional qualities. However, little information is currently available. So, there is an urgent need to focus on improving crops relevant to the small farm holders and poor consumers in the developing countries of the humid and semi-arid tropics (Sharma et al., 2002).

Identifying ecotypes growing under local agricultural conditions with significant levels of beneficial factors could not only provide health benefit to consumers but also promote the value-added cultivation. Biochemical properties could offer reliable tools to distinguish cultivars or ecotypes and may be considered as criteria of selection for specific food applications.

In order to provide information on the composition of pearl millet grown under local conditions, six ecotypes were analyzed to evaluate total phenolic, protein, carbohydrates and reducing sugars content. Polyphenol oxidase (PPO) activity was also investigated and correlated to the total phenolic content and antioxidant potential of each ecotype.

MATERIALS AND METHODS

Plant material

Six autochthonous pearl millet (*Pennisetum glaucum* (L.) R. Br) ecotypes from different ecological regions from Tunisia were studied (Table 1). Three pearl millet populations have tall stature (KS, EC and ZZ) and the others have short stature (HG, AM and D).

Table 1 Pearl millet ecotypes surveyed and their ecological parameters
Tablica 1. Pregled ekotipova bisernog prosa i njihovih ekoloških parametara

Ecotype *Ekotip*	Origin *Porijeklo*	Bio-climatical strata *Bioklimatsko područje*	Annual rainfall *Godišnje oborine* (mm)	Annual average temperature *Srednja godišnja temperatura* (°C)	January average temperature *Prosječna temp. u siječnju* (°C)	July average temperature *Prosječna temp. u srpnju* (°C)
HG	Hamam Laghzaz ; Nabeul	Semi-arid	400-600	18.3	12.1	26.0
AM	El Amra ; Sfax	Arid (Med)	200-400	21.8	11.2	25.9
EC	Echabba ; Mahdia	Arid (Sup)	200-400	19.5	11.9	26.3
KS	Kairouan Sud ; Kairouan	Arid (Med)	200-400	19.9	11.5	28.7
D	Djerba island; Médnine	Arid (Inf)	100-200	21.3	12.4	26.9
ZZ	Zarzis ; Médnine	Arid (Inf)	100-200	22.7	12.7	27.3

Data according to the National Institute of Meteorology (INM), 2006, Tunisia
Podaci Nacionalnog institute za meteorologiju 2006, Tunis

Grains from each ecotype were collected. At the INRAT agronomic station, care was taken to have the same germination and growth conditions for all the grains. After fructification of the cultivated plants, the grains were homogenously collected for a biochemical investigation. In order to keep relatively uniform sample sizes, we limited our random sampling to 20 plants per ecotype. Equal amounts of grains for each ecotype were pooled and ground to a fine powder to obtain the flour.

The experiment was designed as a completely randomized design with one factor.

Protein content

Protein content was determined according to the AOAC procedures (AOAC, 2000).

Nitrogen (N) was analyzed following a colorimetric assay after digestion with sulphuric acid. The % N x 6.25 was used to convert to crude protein.

Total available carbohydrates and reducing sugars content

Available carbohydrates were analyzed after hydrolyzed by sulphuric acid (Ceirwyn, 1995) and optical density at 540 nm was determined by the Spectrophotometer (Miller, 1959).

Glucose was used as standard. Results were expressed as g per 100 g of flour. Reducing sugar analysis was determined by the DNS reagent method.

Total polyphenols content (TPC)

The concentration of total phenolic compounds in water extracts was measured according to Shetty et al. (1995). Supernatant (1 ml) was mixed with 1 ml of 95% ethanol, 5 ml of distilled water, and 0.5 ml of 1N Folin-Ciocalteau reagent and immediately vortexed. After 5 min, 1 ml of sodium carbonate was added and allowed to stand for 60 min in the dark. The samples were mixed again and their absorbances were measured at 725 nm against a 95% ethanol blank. The absorbances were converted to micrograms of catechin per gram of fresh weight of the sample. A calibration curve was generated using (+)-catechin as standard.

Results were expressed as mg catechol equivalents per 100g of flour.

In vitro antioxidant activity

Antioxidant activity of pearl millet flour extracts was determined by DPPH (Sigma Chemical Co.) for radical scavenging following the method described by (Bondet and al., 1979).

The antioxidant activity was expressed as Trolox Equivalent Antioxidant Capacity (TEAC) which was calculated from the equation determined from linear regression after plotting known solutions of the Trolox, used as antioxidant reference, with different concentrations (0.02-0.8 mM).

PPO extraction, preparation and assay

Pearl millet PPO was extracted from grain acetone powder following the procedure described by (Yoruk and Marshall, 2003).

Protein concentration was determined by the Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as a standard.

Enzyme activity was determined spectrophotometrically using catechol as the substrate (Dogan and al., 2005).

Statistical analysis

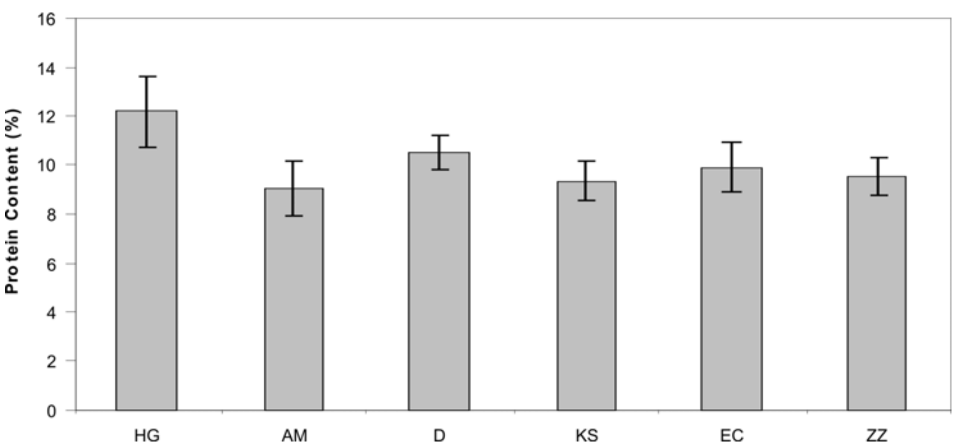
Quantitative presented data are mean ± standard deviation of replicated tests. One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego California USA). The Pearson correlation and regression analysis was performed to determine relationships between different pearl millet contents. Differences of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Varietals variations with respect to chemical composition and some of the technological characteristics of the pearl millet have been recorded (Badi et al., 1976).

The analyses of the protein content of the different samples (Figure 1) showed significant differences among ecotypes (P = 0.9545). Autochthonous pearl millet ecotypes were relatively poor in protein (approximately 10%- 12%). Others researchers have also found that protein content were about 11.8% (Hulse et al., 1980; Barikmo et al., 2004; Lestienne et al., 2007).

Figure 1 Protein content of Tunisian pearl millet ecotypes
Grafikon 1. Sadržaj bjelančevina tuniskih ekotipova bisernog prosa



The highest protein content for HG ecotype could be the result of the use of fertilizer, especially land rotation of pearl millet and leguminous plants in the North of Tunisia.

Protein quantity and quality are affected by a level of nitrogen fertilizer (Doesthale et al.; 1972), and by location and genotype (Ajakaiye, 1984).

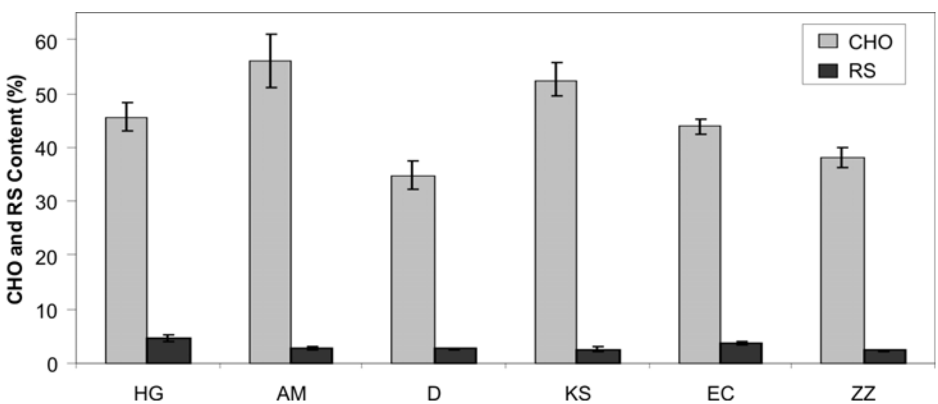
Burton et al. (1972) analyzed pearl millet seeds from 180 inbred lines grown at the same time in a fertilized loamy sand and found protein content in the pearl millet to range from 8.8-20.9% with an average of 16.0%. They concluded that variation in chemical composition for cereals in general is expected depending on genotype and the environment in which the cereal is grown. Factors such as soil moisture content, level of nitrogen in the soil and the time of nitrogen fertilizer application also influence protein content of cereals.

Cereal grains are generally known to contain high levels of carbohydrates. Investigated pearl millet grains in the present work showed a total carbohydrate content ranging from 34.78 % to 56.07 % (Figure 2). Similar result was obtained for malian pearl millet (Barikmo and al., 2004).

The six pearl millet ecotypes carbohydrates exhibit substantial differences with respect to their composition. In particular AM ecotype is about twice as rich in carbohydrates compared with D ecotype.

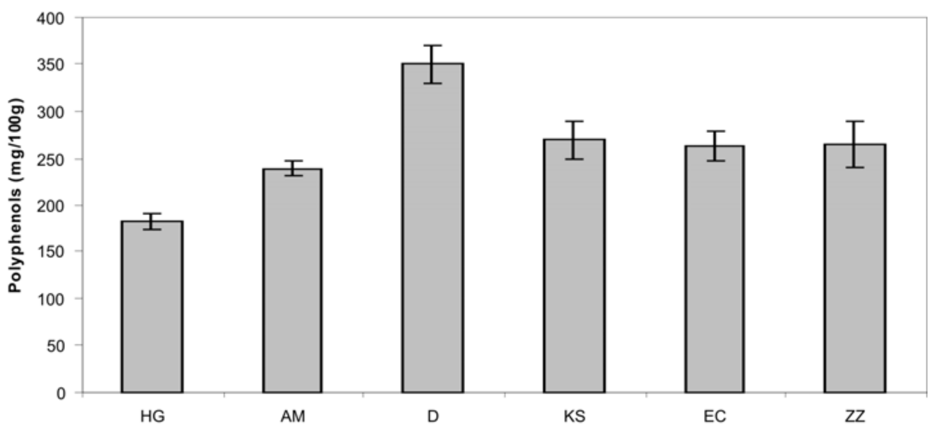
Reducing sugars content of the Tunisian pearl millet grains represented 7 % of the total available carbohydrate suggesting a high percentage of oligo- and polysaccharides. The present findings are in agreement with those reported by Lestienne and al. (2007).

Figure 2 Total carbohydrate (CHO) and reducing sugar (RS) contents of Tunisian pearl millet ecotypes
Grafikon 2. Sadržaj ukupnih ugljikohidrata i reducirajućih šećera tuniskih ekotipova bisernog prosa



The level of total phenolic compounds in the Tunisian pearl millet flours ranged from 181.8 to 349.8 mg catechol equivalent per 100 g of fresh weight (Figure 3). D ecotype identified as containing the highest phenolic content was originating from the south of Tunisia. In these regions, pearl millet grows under difficult ecological conditions compared to those cultivated in the north. The fact that polyphenolics are believed to be involved in plant protection against UV radiation and resistance to pathogens and predators (Charanjit and Kapoor, 2001) could explain, in part, the high phenolic content of D ecotype.

Figure 3 Polyphenolic content of Tunisian pearl millet ecotypes
Grafikon 3. Sadržaj polifenola tuniskih ekotipova bisernog prosa



The lowest value was recorded for HG ecotype grown in the north. Thus, it seems that the phenolic content depends on the geographical origin of the ecotype.

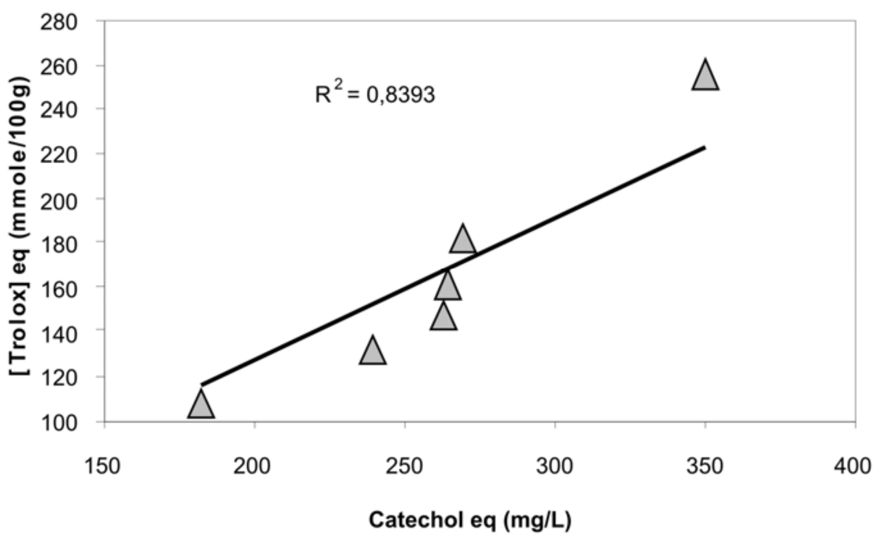
In general, ferulic, p-coumaric, and cinnamic acids are the major phenolic acids in millets (McDonough et al., 1986; McDonough and Rooney, 2000). These phenolic acids are located in the pericarp, testa, aleurone layer, and endosperm (Hahn et al., 1984; McDonough et al., 1986).

Phenolic content, as some other biochemical constituents, was shown to be determinants in food quality (Thomas-Barberan and Espin, 2001). Regarding the favourable redox potentials and the relative stability of their phenoxyl radical, these biomolecules are considered as the main human health promoting antioxidants (Acuna and al., 2002). Many naturally occurring phenolics, in particular those found in cereal grains, exhibited potential antioxidant activities *in vitro* and *in vivo* (Charanjit and Kapoor, 2001). In the present work, we investigated the *in vitro* antioxidant capacity of the pearl millet grain phenolic extracts. The radical scavenging capacities against DPPH of all the ecotypes were found relatively high. Our results were in accordance with recently reported findings in sorghum grains (Dykes and al., 2005). D ecotype from Djerba exhibited the highest antioxidant activity. So, it could be considered as good source of antioxidants and could be used in the functional food/nutraceutical industry. This is the first report about the antioxidant activity of this cereal in Tunisia.

A correlation analysis was made between polyphenolic content and antioxidant potential of all the Tunisian pearl millet ecotypes (Figure 4). The two parameters were found very closely correlated for the set of all the samples (polyph/antiox and r = 0, 923773; $P < 0.0001$).

Our data indicate that the antioxidant power in the pearl millet extracts is determined, or highly influenced, by their polyphenolic content. Significant positive correlations between these two parameters were also reported in numerous analytical studies (Alonso and al., 2004; Dykes and al., 2005,).

Figure 4 Correlation between total polyphenolic content and DPPH scavenging capacity of the millet grain extracts
Grafikon 4. Korelacija između ukupnog sadržaj polifenola i DPPH ionizacijskog kapaciteta ekstrakta zrna prosa



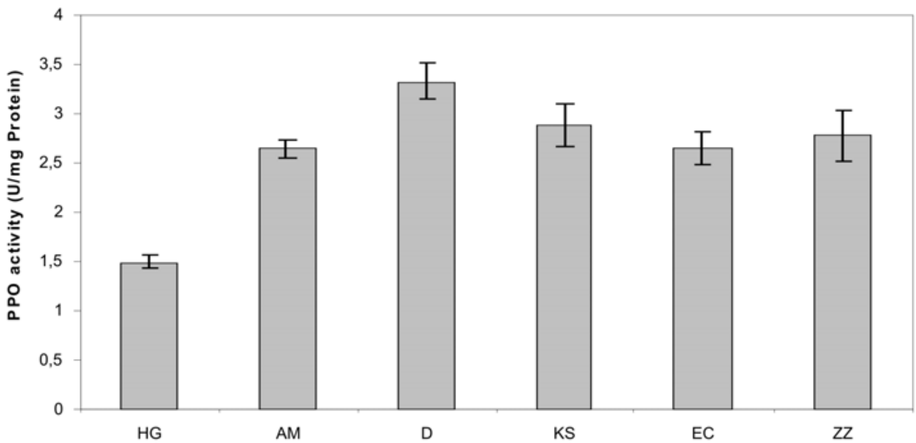
Mean of five replications was considered for each ecotype
Prosjek petero ponavljanja za svaki ekotip

Using Catechol as substrate, the PPO specific activity of the analysed pear millet grains was ranged from 1.4 to 3.3 U/mg of protein (Figure 5) and significant differences among the analysed ecotypes were found ($P < 0.0001$). This result was corroborated by several authors (Dykes and al., 2005).

For many cereals, it was shown that genetic factors could be responsible for total phenolic contents and oxidative enzymes differences among varieties (Thomas-Barberan and Espin, 2001).

In addition to a cultivar effect, numerous studies on wheat have shown that PPO activity was also affected by environmental factors (Chan and al., 1978; Demeke and al., 2001). The observed diversity among Tunisian ecotypes could be explained mainly to the farmer's selection of adapted parents to local environmental conditions. In fact, it was reported that variation in wheat flour PPO activities among growing locations was greater than variation among genotypes (Park and al., 1997).

Figure 5 PPO specific activity of Tunisian pearl millet ecotypes
Grafikon 3. PPO specifična aktivnost tuniskih ekotipova bisernog prosa



In order to assess the correlations between the different analysed components of the six pearl millet ecotypes, Pearson correlation coefficient was calculated. The result showed that polyphenol content, antioxidant potential and PPO activity were positively correlated (0.9328 for PPO/polyph and 0.8375 for PPO/Antiox; $P < 0.0001$). The PPO seems to improve the protective effects of polyphenols. In fact, oxidative enzymes are known to play an important role in plant defence by the oxidation of endogenous phenolics into their corresponding quinones, which are toxic to the invading pathogens and pests (Chan and al., 1978). The resulting quinones may undergo nonenzymatic autopolymerization or covalent hetero-condensation with proteins and carbohydrates to constitute a physical barrier against biotic and abiotic stresses (Chan and al., 1978).

CONCLUSION

In this study, differences in macro-nutrients content, mainly total polyphenols and PPO activities, among Tunisian pearl millet ecotypes were found. Such information would be of use to identify cultivars suitable for various end uses and assist the plant breeders to improve the grain quality.

Biochemical properties may be used as criteria of selection for a specific food application. For example, the oxidation of endogenous polyphenols with a relatively strong PPO activity for D ecotype may yield a desired opaque colour of the millet flour and influence its organoleptic properties. So, this ecotype could be suitable for an industrial application where high enzymatic browning is required. In addition, some ecotypes showed great antioxidant level and could be potentially quite important sources of health promoting foods. Since radical scavenging potential has been shown to depend on the phenolic content, our study can be further extended to assess the identification of the phenolic compounds.

BIOKEMIJSKA ISTRAŽIVANJA TUNISKIH AUTOHTONIH EKOTIPOVA BISRNOG PROSA (*Pennisetum glaucum* (L.) R. Br)

SAŽETAK

Biserno proso (*Pennisetum glaucum* (L.) R. Br) osnovna je hrana u mnogim zemljama u razvoju i predstavlja najveći izvor osnovnih hranidbenih sastojaka u polu-pustinjskim i pustinjskim regijama Afrike i Indije. Znanje o nutricionističkom sastavu autohtonih ekotipova bisernog prosa je površno. U ovoj studiji obrađena je biokemijska analiza šest tuniskih ekotipova bisernog prosa. Rezultati dokazuju razlike u količinama polifenola, antioksidans potencijala i polifenol oksidacijske (PPO) aktivnosti unutar proučavanih ekotipova. Utvrđeno je da su ova tri parametra međusobno povezana. Štoviše, neki ekotipovi mogli bi se iskoristiti kao kriterij selekcije za određenu primjenu u prehrani, pogotovo pri korištenju ove žitarice u raznim prehrambenim proizvodim.

Ključne riječi: *Pennisetum glaucum*, ekotip, biokemijski sastav

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