

Phenolic, Flavonoid and Tannin Contents of Tunisian Barley Landraces

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Abstract: There is a renewed interest throughout the world in barley food because of its nutritional value. Barley is a rich source of various phytochemicals, including phenolic acids, flavonoids and tannins. Natural compounds in food are an important health protecting factor. In Tunisia, many autochthonous barley landraces have generated interest because their nutritional qualities. In order to provide information on the composition of barley grown under local conditions, 37 landraces were analyzed to evaluate their nutraceutical properties. The nutraceutical properties were determined by evaluating TPC, TFC, TCT. The results showed that and the total phenolic varied widely between ecotypes (from 70 to 195 mg GAE/100 g). The TFC varied from 47 to 123 (mg CE/100g flour) and TCT ranged from 9 to 70 (mg CE/100g flour).

Identifying barley landraces 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 and stimulate industrial and economic growth.

Keywords: Barley, Landrace, Phenolic, Flavonoid, Tannin.

I. INTRODUCTION

In addition to being primary sources of carbohydrates, barley also provide trace minerals, dietary fibre and bioactive compounds [1], [2].

Barley grains have been investigated for several potential new applications as a whole grain for its value-added product [3]. There is a renewed interest throughout the world in barley food because of its nutritional value [4]. In fact, grains contain beneficial ingredients for health which reduced risk of developing chronic diseases such as type 2 diabetes and cardiovascular disease, gastrointestinal cancer [5],[6],[7], hypercholesterolemia, hypercalcaemia, and kidney stones [8], [9]. These health benefits have been partly attributed to the wide variety of potential chemopreventive substances, called phytochemicals, such as polyphenols (phenolic acids and their derivatives), flavonoids and tannins present in high amounts in such foods [10], [11], [12].

The recent surge in interest in phenolic compounds in plants is also due to their antifungal effect [13], [14].and to prevent invading pathogens and pests [15]. Cereal grains with elevated levels of phenolic acids in caryopsis exhibit

greater resistance to both disease and insect [16], [17], [18].

Several studies in many species have shown that the levels of phenolic compounds, are different between resistant plants and plants susceptible to stresses [15], [19]. An increase in the activities of phenolic related enzymes and the accumulation of phenolic compounds have been correlated with resistance of cereals to biotic stresses[15]. Plant resistance to biotic and abiotic stresses is often regulated by the metabolism of phenolic compounds [20],[21]. Polyphenol quantity and quality in plant foods can vary significantly according to different intrinsic and extrinsic factors such as plant genetics and cultivar, soil composition and growing conditions, maturity state and post harvest conditions, among others [22].

In Tunisia, barley is cultivated in all regions of the country and occupied between 34% and 38% of the cereal cultivated area [23]. It is used both as feed and food. Over time, local knowledge presented barley as a high energy and healthy food that is used in a diversity of recipes and rooted in the culture and tradition of people's diet.

Tunisian local landraces are preferred for food preparations rather than improved cultivars [24] and most farmers are attached to their local germoplasm [25]. These landraces are distinguished by farmers who usually gave them a name that is associated with their specific traits (in cereals, these often refer to the spike and kernel characteristics), and different farmers might use the same landrace with the cultivation of different populations. The process of agricultural industrialization was associated with the loss of many of the landraces that were reproduced on farms through farmer selection of seed for the planting of the next generation [26]. In some marginal areas, the cultivation of landraces still continues, where farmers can take advantage of the specific adaptations of these landraces to the agro-ecosystem, for the preparation of typical products, and for cultural and religious reasons [27].

Genetic diversity provides an ample opportunity to identify and breed barley varieties for specific end uses. Up to today, the local genetic resources of Tunisian barley received only a modest benefit compared with improved varieties. Identifying landraces growing under local agricultural conditions with significant levels of beneficial

factors could not only provide health benefit to consumers but also promote value-added cultivation and stimulate industrial and economic growth. According to several authors, knowledge about any germplasm diversity and genetic relationships could be an invaluable aid in crop improvement strategies [28]. Thus, criteria for genetic diversity estimation can be different: pedigree records, morphological traits, biochemical markers and molecular markers [29]. Biochemical properties also are key tools in the evaluation of genetic variability in both natural populations and germplasm accessions [30]. It could offer a reliable tools to distinguish cultivars or landraces and may be considered as criteria of selection for specific food applications [31].

The aim of the present study was to compare the levels of endogenous phenolic compounds in barley grain for resistance or susceptibility to biotic and abiotic stresses and for the selection of landraces with potentially positive health benefits compared with improved varieties.

II. MATERIALS AND METHODS

A. Plant material

34 local landraces barley (*Hordeum vulgare* L.) were collected from different ecological regions from Tunisia and 3 improved varieties were obtained from Crops Laboratory of the National Institute of Agronomic Research of Tunisia (table 1). All of them were six-rowed spring barley. Each landrace or variety is affected of an abbreviation symbolizing the zone of origin.

B. Plant growth and treatments

Collected grains from each landrace were sowed at the farm of the Tunisian Agricultural Research Institute in Béja during the 2013 cropping season. The soil of the experimental site was clay loam. They were sown on 15 November 2013 into randomized block design with four replications. After fructification of the cultivated plants, the grains were homogeneously 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.

C. Reagents

Folin-Ciocalteu's phenolic reagent were obtained from Sigma-Aldrich Co. Authentic standards of phenolic compounds were purchased from Sigma and Fluka. All other chemicals used were of analytical grade.

D. Extraction of samples

2,5g of grain barley powder were extracted by stirring 25 ml of Ethanol 70% at room temperature for 30 minutes. Extracts were kept for 24 h at 4°C, and then filtered through Whatman filter paper and evaporated to dryness under vacuum. They were stored at 4° C until analysis.

E. Phenolic compound analysis (TPC)

Total phenolics of barley extract were determined using the Folin-Ciocalteu reagent according to the method described by [32]. An aliquot of 0,125 ml of diluted sample was added to 0,5 ml of distilled water and 0,125 ml

of 7% Na₂CO₃. The volume obtained was adjusted to 3 ml using distilled water, mixed vigorously, and incubated in the dark for 90 min at ambient temperature. The absorbance of the solution was then measured at 760 nm versus the prepared blank. The total phenolic content was expressed as mg of gallic acid equivalents per 100 g flour (mg GAE /100g) through the calibration curve with gallic acid. All samples were analyzed in three replications.

F. Total flavonoid content (TFC)

Total flavonoids were measured by colorimetric assay according to [32]. 250µl of extract was mixed with 75µl NaNO₂ (5%). After 6min, we added 150 µl of 10% AlCl₃ and 5min later, 500µl of NaOH (1M) was added to the mixture. The final volume was adjusted to 2, 5 ml with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid content of seeds was expressed as mg catechin equivalents/ g dry weight (mg CE/g DW), through the calibration curve with catechin. The calibration curve range was 0-400 µg/ml.

G. Total condensed tannins (TCT)

In presence of H₂SO₄, condensed tannins were transformed by the reaction with vanillin [33]. An aliquot (50µl) of each extract or standard solution was mixed with 1,5 ml of 4% methanol vanillin solution and then 750 µl of concentrated HCl were added. The well mixed solution was allowed to stand for 15 min, and the absorbance was measured at 500 nm. The amount of total condensed tannins were expressed as mg catechin equivalent/g dry weight. All samples were analyzed in three replications.

H. Statistical Analyses

All tests were conducted in triplicate. Analysis of variance and significant differences among means were tested by one way ANOVA using Statistical Analysis System (SAS), version 9 software. Correlations were done using Pearson coefficient correlation.

III. RESULTS AND DISCUSSION

Barley vary widely in their phenolic composition and content, with both genetics and environment affecting the kind and level of phenolic compounds.

A. Total phenolic content (TPC)

Phenolic acids are presents in cereal grains [34], [35] and their content in cereals is usually less than 1% of dry matter, except for some sorghum cultivars [36] and finger millet varieties [37]. Benzoic and cinnamic acids-derivatives are the two main groups of phenolic acids present in cereal kernels [38]. The outer layers of the barley grains are characterized by having relatively high concentrations of phenolic compounds, such as lignin, lignans, flavonoids, and ferulic and coumaric acids [39], [40], [41]. It was reported that barley contains more total phenols than hard and soft wheat, but less than millet, rye, and sorghum [42]. The purple and blue colours in barley kernels are usually the results of a phenolic compound on the surface of the grains, particularly the anthocyanins [43]. According to [44], aleurone, bran and whole grain significantly differed in their phenolic acids composition

and antioxidant properties, with the aleurone layer the richest portion.

Total phenolic content (TPC) varied significantly among barley landraces and varieties (tab.1). The highest TPC was observed in local landrace Djerba cultivar (DJ5) with 195 mg GAE/ 100g, while OG2 ecotype from oasis Gabes showed the lowest TPC value (71 mg GAE/100g). Barley landraces from Djerba and Mahdia contained more phenolic content than others barley. Instead, landraces from oasis have low TPC content. The TPC of whole flour from autochthonous barley varied significantly among the landraces but it is comparable to Czech barley landraces which polyphenol content varied between 60 and 150 mg/100g [40].

Some authors [45], [46] reported TPC of some barley varieties to be from 45 to 135 mg/g mg GAE/100g flour. Total polyphenols content varied from 100 to 450mg/100g for twelve barley cultivars native from USA [47].

[48] found TPC varies within the range 114 -227 mg/100g for Italian barley. However, for twenty Japanese barley landraces, TPC varied from 154 to 324 mg/100g.

Several factors affect the phenolic content and availability, including environmental conditions, agronomic practices [49], [50] and industrial or domestic processing [51].

B. Total Flavonoid Content (TFC)

Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plant. They provide colouring to various tissues including flowers [52], [53]. These compounds serve essential functions in plant reproduction by recruiting pollinators and seed dispersers [54]. There are potent antioxidants, free radical scavengers [55] and metal chelators and inhibit lipid peroxidation [56]. Flavonoids are also produced inducibly by plants during stress, ostensibly as a natural defence mechanism [57], [58]. Flavonoids are reported to have antioxidant, anticancer, anti allergic, anti-inflammatory, anticarcinogenic and gastro protective properties [59], [60], [61].

Cereals have only small quantities of flavonoids [62], except barley that contains measurable amounts of catechin and some di and tri pro-cyanidins [63], [64].

Total flavonoids content (TFC) varied significantly among autochthonous barley landraces and varieties (tab.1). In fact, TFC in several ecotypes (KK1, KK2, and M5) were the highest when compared to the others landraces. The lowest flavonoid level was presented by Kounouz variety and landraces from Gabes oasis (less than 50 mg CE/100g flour). These results were lower than those obtained by [65] for Rihane and Manel tunisian varieties barley but higher than those reported by [66]. Other works [67] had noted that Korean barley flavonoids content varied from 77 to 163µg/g.

For Dutch barley, [68] reported 26.9 mg/100g of flavonoid content. These differences in barley flavonoid contents may be due to barley samples and TFC evaluation methods used in these studies. [69] reported that the levels of flavonoids in fruits and vegetables are influenced by genotype (cultivar or variety), agronomic practices

(irrigation, fertilization, and pest management), climatic and regional conditions (temperature, light, moisture, and ultraviolet radiation), maturity at harvest, postharvest handling, and storage conditions.

We have found that ecotypes from oasis had low content of flavonoid. This may be due to a specific micro climate of the oasis: a lot of shade (less radiation), freshness and moisture. At the population level, there was considerable variation in secondary metabolite responses to UV-B radiation [70]. It has been proposed that differences in UV-B radiation along latitudinal or elevational gradients have resulted in plant species from diverse UV-B habitats developing contrasting sensitivities to UV-B radiation [71], [72], [73]. There were also some UV-B induced differences in the responses of populations from different geographical locations [74]. Many research demonstrated also that flavonoids increase more in stress-sensitive species than in stress-tolerant species [75], [76].

C. Total condensed tannin (TCT)

Tannins are phenolic plant secondary compounds [77] with ranging molecular weights and complexity [78],[79]. They are widely distributed in nature and are the fourth most abundant plant constituent following cellulose, hemicellulose and lignine [80]. Tannins have several specific properties: antibacterial, antitumor, antiviral and antimutagenic functions [81]. Plant tannins have also beneficial properties to human health [82].

Tannin are present in two distinct types: hydrolysable tannins (HT) and condensed tannins (CT). The value of CT depends on the analytical technique [83] and the plant species [84].

Important grains which are used for human and animal consumption are known to contain a significant amount of tannin contents like sorghum (*Sorghum bicolor*), millet (*Panicum milisceum*), barley (*Hordeum vulgare*) and a number of other legume seeds [85],[86],[87],[88]. Furthermore, condensed tannin concentration in plant tissue has been shown to vary with species, plant part, plant maturity, drought, CO₂ concentration and soil fertility [89],[90].

The TCT of whole flour from autochthonous barley varied significantly among the landraces. Total condensed tannins varied significantly among barley landraces and varieties (tab.1). The highest TCT was observed in local landrace Djerba cultivar (DJ8) and 2 ecotypes from Mahdia (M1 and M3) with about 70 mg CE/ 100g flour, while ecotypes from oasis Gabes and Gabes showed the lowest TCT values (between 9 and 13 mg CE/100g). Barley landraces from Djerba and Mahdia contained more tannin contents than others barley. Instead, landraces from Gabes and especially Gabes oasis have low TCT content.

Tunisian barley landraces are more rich in tannin than Kenyan varieties which contain 10 to 12 mg CE/100g [91].

There is a well-established positive relationship between the intensity of solar radiation and the quantity of phenolics produced by plants. Generally there is a rise in total phenolics in plants grown in sunny situations relative to shady ones [92]. TCT also increased at high temperatures [89],[93].

IV. CONCLUSION

Since barley is the rich source of antioxidants, the varieties with high activity will be preferred for malt preparation. For food purpose also varieties with high antioxidant activity will provide more health benefits. The sources identified for high antioxidant activity can be used in the malt and food barley improvement programme of the country to develop varieties with higher anti-oxidant activity. Also, barley grains with elevated levels of phenolic acids in caryopsis are more resistant to biotic and abiotic stresses, so they may be used in research programs to a more appropriate zoning especially in the current context of climate change.

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Table 1. Accessions' origin, bioclimatic stage and rainfall (Data according to the National Institute of Meteorology (INM), 2014, Tunisia.

Accession	Abbreviation	Origin	Bioclimatic stage	Rainfall (mm)
Oasis Gabes 1	OG1	Gabes	Arid inf	100-150
Oasis Gabes 2	OG2	Gabes		
Oasis Gabes 3	OG3	Gabes		
Kerkennah 1	KK1	Kerkennah island	Arid inf	<250
Kerkennah 2	KK2	Kerkennah island		
Kerkennah 3	KK3	Kerkennah island		
Kerkennah 4	KK4	Kerkennah island		
Kerkennah 5	KK5	Kerkennah island		
Kerkennah 6	KK6	Kerkennah island		
Kerkennah 7	KK7	Kerkennah island		
Djerba 1	DJ1	Djerba island	Arid sup	100-200
Djerba 2	DJ2	Djerba island		
Djerba 3	DJ3	Djerba island		
Djerba 4	DJ4	Djerba island		
Djerba 5	DJ5	Djerba island		
Djerba 6	DJ6	Djerba island		
Djerba 7	DJ7	Djerba island		
Djerba 8	DJ8	Djerba island		
Djerba 9	DJ9	Djerba island		
Djerba 10	DJ10	Djerba island		
Djerba 11	DJ11	Djerba island		
Mahdia 1	M1	Mahdia	Semi arid inf	200-400
Mahdia 2	M2	Mahdia		
Mahdia 3	M3	Mahdia		
Mahdia 4	M4	Mahdia		
Mahdia 5	M5	Mahdia		
Mahdia 6	M6	Mahdia		
Mednine 1	MD1	Mednine	Arid inf	100-200
Mednine 2	MD2	Mednine		
Mednine 3	MD3	Mednine		
Kairouan 1	KR1	Kairouan	Arid sup	200-400
Kairouan 2	KR2	Kairouan		
Gabes 1	G1	Gabes	Arid inf	100-150
Gabes 2	G2	Gabes		
Kounouz	KOU	Tunisian barley improved variety,		

Manel	MAN	registered in 2011 Tunisian barley improved variety, registered in 1996		
Rihane	RIH	Tunisian barley improved variety, registered in 1987		

Table 2. Phytochemical contents of Tunisian barley varieties and landraces

Accession	Abbreviation	TPC (mg GAE/100g flour)	TFC (mg CE/100g flour)	TCT (mg CE/100g flour)
Oasis Gabes 1	OG1	75.04 g	49.67 g	10.66 g
Oasis Gabes 2	OG2	70.99 g	47.05 g	9.00 g
Oasis Gabes 3	OG3	74.96 g	49.17 g	13.16 g
Kerkennah 1	KK1	179.86 b	121.69 a	46.50 d
Kerkennah 2	KK2	180.07 b	117.65 a	36.50 e
Kerkennah 3	KK3	182.84 b	78.86 d	38.16 e
Kerkennah 4	KK4	140.64 c	69.57 e	36.50 e
Kerkennah 5	KK5	178.44 b	54.02 f	33.16 e
Kerkennah 6	KK6	120.99 d	85.33 c	44.83 d
Kerkennah 7	KK7	119.15 d	88.36 c	40.66 d
Djerba 1	DJ1	193.40 a	71.39 d	61.50 b
Djerba 2	DJ2	180.71 b	73.11 d	50.66 c
Djerba 3	DJ3	194.89 a	99.37 b	62.33 b
Djerba 4	DJ4	190.28 a	73.71 d	56.50 c
Djerba 5	DJ5	195.11 a	80.48 c	64.83 b
Djerba 6	DJ6	194.68 a	100.28 b	62.33 b
Djerba 7	DJ7	194.18 a	90.08 b	63.16 b
Djerba 8	DJ8	195.04 a	97.65 b	70.66 a
Djerba 9	DJ9	181.06 b	61.69 e	56.50 c
Djerba 10	DJ10	181.77 b	61.09 e	49.00 c
Djerba 11	DJ11	192.27 a	76.54 d	54.00 c
Mahdia 1	M1	192.91 a	83.31 c	70.66 a
Mahdia 2	M2	192.62 a	95.33 b	65.66 b
Mahdia 3	M3	192.70 a	82.30 c	68.16 a
Mahdia 4	M4	192.41 a	88.86 c	60.66 b
Mahdia 5	M5	193.12 a	123.71a	60.66 b
Mahdia 6	M6	193.90 a	84.32 c	66.50 b
Mednine 1	MD1	120.21 d	65.23 e	50.66 c
Mednine 2	MD2	122.06 d	50.68 f	49.83 c
Mednine 3	MD3	111.28 e	56.44 f	45.66 d
Kairouan 1	KR1	90.71 f	59.27 f	28.16 f
Kairouan 2	KR2	90.00 f	59.07 f	24.83 f
Gabes 1	G1	145.82 c	50.48 f	11.50 g
Gabes 2	G2	145.04 c	50.48 f	20.66 f
Kounouz	KOU	71.84 g	43.41 g	25.66 f
Manel	MAN	116.6 e	73.01 d	35.66 e
Rihane	RIH	182.98 b	103.21 b	51.50 c

Values in the same column not sharing a common letter (a-g) differ significantly at $p < 0.05$.

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