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Bioactive Compounds, Antioxidant Activity and Growth Behavior in Lettuce Cultivars Grown under Field and Greenhouse Conditions †

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Abstract: Lettuce (*Lactuca sativa* L.) is one of the most important leafy greens worldwide. The nutritional value of its edible leaf depends on different factors including type and growing conditions. The aim was to determine the bioactive compounds content, antioxidant activity and growth behavior of twenty-two lettuce genotypes, cultivated under field and greenhouse conditions. Total phenolic compound, chlorophylls, carotenoids, anthocyanin contents and antioxidant activities were analyzed by spectrophotometric methods. Data were analyzed by analysis of variance (ANOVA). Significant differences between bioactive compounds, antioxidant activity and growth behavior were found among cultivars and morphological types, for both growth conditions. Carotenoid and chlorophyll content was higher in greenhouse conditions for all genotypes. In field production, butterhead and iceberg lettuces showed lower content of these bioactive compounds. The red-pigmented Falbala cultivar from field production showed the highest level of polyphenols and anthocyanin. Meanwhile, in greenhouse conditions, the oak leaf cultivar Grenadine displayed the highest concentration of these phenolic compounds. The iceberg type lettuce showed the lowest percentages of antioxidant activity in both environments. The results showed the effect of growing conditions and the high variability in lettuce bioactive compounds content and antioxidant activity among the different types.

Keywords: *Lactuca sativa* L.; production systems; phytochemical compounds; biological properties

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1. Introduction

In recent decades, consumers have an increasing interest in healthier and safer food. Nutrition quality and bioactive compounds in the different food sources have been widely studied, focusing on the contribution of phytochemical consumption on human health. Lettuce, *Lactuca sativa* L., is one of the most popular leafy vegetables, widely consumed in salads, ready to eat products and baby leaf mixes. This species has a huge morphological variability, which has led to the classification into several types. The color, size, texture and taste are important parameters for successful marketing of lettuce and these factors determine the market price and the consumer preference. Lettuce is mostly consumed in raw, that makes it a good source of dietary fiber, vitamin A, folate, vitamin C, vitamin K and phenolic compounds [1,2]. Daily consumption of lettuce has been associated with the promotion of human health and reduction of the incidence of chronic diseases [3,4]. Several factors affect the concentration of bioactive compounds including genotype, agroclimatic conditions, light radiation, postharvest handling, etc. [5–8]. Therefore, the objective of this work was to study the interaction between genotype and environmental conditions regarding bioactive compound content, antioxidant activity and growth behavior of twenty-two lettuce genotypes, cultivated under field and greenhouse conditions.

2. Materials and Methods

2.1. Plant Material

Twenty-two lettuce genotypes, including commercial cultivars and inbreeding lines (BL), were selected for this study (Table 1 and Table S1). The experiment was conducted in INTA's experimental field located in La Consulta, Mendoza, Argentina (33°44′ S, 69°07′ W) during winter season, using a complete randomized design. The seedlings were transplanted in June with a density of 13 plants·m^{-2}, under two cropping systems: field and greenhouse production. The soil texture was sandy loam in both environments. The average temperature ranked from 2.5 °C to 19.7 °C for all the season on field production. For the greenhouse, the temperature was slightly higher during all the lettuce production. Once they reached commercial size, ten plants per cultivar were harvested and used for analytical determinations.

Table 1. Lettuce genotypes classified according to L'Union Internationale pour la protection des obtentions végétales (UPOV).

Type	Genotypes
Iceberg	83-25-317; Dessert storm; BL001; BL003; Road runner; Valley Green
Crisped head	Bacchus; Falbala; BL009; BL010; BL011; Lírice
Batavia	Rossia
Oak leaf	Grenadine
Latin	Crimor; Maravimor
Butterhead	Balerina; BL006; Lores
Romaine	BL012; BL013; BL014

2.2. Biometric Measurements

For biometric measurements, stem diameter, plant height and fresh plant weight were determined for 10 plants per cultivar for each cropping system. The color was measured using a colorimeter (CR-400-Konica Minolta) equipped with a D65 illuminant in the reflectance mode and in the CIE L* a* b* color scale. In detail, L* indicates the lightness from black (0 value) to white (100 value), a* the redness (+) or greenness (−), and b* the yellowness (+) or blueness (−).

Chroma and hue angle were calculated as follows:

$$C = \sqrt{a^2 + b^2} \qquad h^\circ = \arctan\frac{b}{a}$$

2.3. Bioactive Compounds and Antioxidant Activity

The total phenolic compounds were measured using the Folin-Ciocalteu method [9]; carotenoid and chlorophylls content were determined following the methodology of Lichtenthaler and Buschmann, 2001 [10] (pp. F4.2.1–F4.2.6) and anthocyanin contents were determined using a spectrophotometric pH-differential method [9].

The antioxidant activity of the extract was determined according to Soto et al., 2016 [11] (pp. 277–289), using, 2.2-diphenyl-1-picril-Hidrazil (DPPH). Samples were measured (A_E) against methanol and methanol with a DPPH· blank (A_B). The experiment was carried out three times and the absorbance sample (A_0) was considered. A pyrogallol solution (1 mM) was used as a reference (A_{REF}). Radical scavenging activity was calculated in percentage of inhibition (I%) as follows:

$$I\,(\%) = \left[\frac{A_B - (A_E - A_0)}{A_B} \Big/ \frac{A_B - A_{REF}}{A_B}\right]$$

2.4. Statistical Analysis

The data were analyzed with STATISTICA 10 (Statistica 7; StatSoft, Tulsa, OK, USA) package. The effects of the production system and the cultivar on the bioactive compounds, antioxidant activity and growth behavior were evaluated by analysis of variance (ANOVA), followed by the post-hoc Tukey's HSD test ($p < 0.05$). Principal component analysis was performed in order to explore the variation and identify potential patterns within the dataset.

3. Results and Discussion

In the present study, bioactive compound content, antioxidant activity and growth behavior were analyzed from twenty-two lettuce genotypes, cultivated under field and greenhouse conditions. The results from growth parameters are presented in Table 2. Significant differences were observed in stem diameter, height and fresh weight among plants under both cropping systems. Batavia showed higher stem diameter in field production compared to greenhouse production (on average 3.27 and 1.25 cm, respectively). Romaine and butterhead showed the lowest and highest plant height in the greenhouse (22.53 and 36.94 cm, respectively). In general, the lettuce types cultivated under field production reached higher fresh plant weight than the types cultivated in greenhouse.

Table 2. Stem diameter, height and fresh weight plant of 7 types of lettuces in two cropping systems.

Type Cultivar	FIELD PRODUCTION		
	Stem Diameter (cm)	**Height Plant (cm)**	**Fresh Weight Plant (g)**
Iceberg	1.67 ± 0.94 [1] a [2]	32.67 ± 1.81 cd	465.45 ± 48.20 f
Crisped head	1.89 ± 0.68 ab	26.87 ± 4.65 ab	299.81 ± 89.57 cde
Batavia	3.27 ± 1.10 b	27.67 ± 1.53 abc	436.57 ± 78.95 ef
Oak leaf	2.57 ± 0.25 ab	30.00 ± 1.00 abcd	251.40 ± 15.81 abcde
Latin	2.28 ± 0.45 ab	31.83 ± 5.42 bcd	362.83 ± 42.36 def
Butterhead	1.61 ± 0.18 a	28.38 ± 5.74 abc	326.22 ± 94.90 de
Romaine	2.46 ± 0.24 ab	27.39 ± 3.53 ab	479.03 ± 58.93 f
	GREENHOUSE PRODUCTION		
	Stem Diameter (cm^2)	**Height Plant (cm)**	**Fresh Weight Plant (g)**
Iceberg	1.85 ± 0.99 ab	29.22 ± 2.67 bc	209.48 ± 75.97 bc
Crisped head	1.87 ± 0.59 ab	34.31± 20.71 ab	96.11 ± 39.53 a
Batavia	1.50 ± 0.30 ab	22.62 ± 2.09 abcd	16.,09 ± 61.19 abcd
Oak leaf	2.60 ± 0.10 ab	22.62 ± 3.07 ab	39.35 ± 6.11 ab
Latin	2.58 ± 0.94 ab	31.07 ± 1.79 abcd	140.21 ± 4.90 ab
Butterhead	1.43 ± 0.67 a	36.94 ± 4.40 d	179.79 ± 58.89 ab
Romaine	2.82 ± 0.39 b	22.53 ± 3.16 a	111.68 ± 22.62 ab

[1] Mean ± SD. [2] Values in the same column marked by different letters differ statistically ($p < 0.05$).

Color is also an important factor of lettuce market quality. Color parameters are strongly influenced by genetics, environmental factors, such us, light/radiation and temperature [12,13]. Our results showed that the production system affected the color of leaves and there were significant differences in terms of chroma (C*), lightness (L*) and hue (h°) values (Table S1). We observed that lettuce leaves produced under greenhouse were characterized by more intense green colors, while lettuce produced in a field shown more intensity of red tone, resulting in products with higher chromaticities that are more attractive for consumers.

The bioactive compounds showed significant differences among cultivars from the same type ($p < 0.05$). The levels of total chlorophylls in field production varied within a broad range 0.67–2.02 mg·g^{-1} dw, while in greenhouse they varied within 0.86–1.67 mg·g^{-1} dw. Oak leaf type had the highest contents of chlorophylls a, chlorophylls b and carotenoids (Table S2). In addition, oak leaf type presented the highest total anthocyanins content (7.22 mg·g^{-1} DW) and total phenolic compounds (29.21 mgEAGIg^{-1}). On the other hand, Latin type showed the lowest levels of these bioactive compounds in both production systems (Table S2). The red-pigmented Falbala cultivar from field production showed the highest level of polyphenols and anthocyanin. Meanwhile, from greenhouse conditions, the oak leaf cultivar Grenadine had the highest concentration of these phenolic compounds (Table S3).

Previous studies have reported that several factors influence the bioactive compounds content [14–16], the most important of which include factor season, temperature, water availability, mechanical damage and attack of pathogens. Our results show that the differences found in the bioactive compound content could be due to agricultural practices adopted in each production system.

The relative composition of bioactive compounds in both production systems is shown in Figure 1. In field production, lettuce showed higher levels of phenolic compounds and anthocyanins, while in greenhouse production lettuce showed higher levels of chlorophylls and carotenoids.

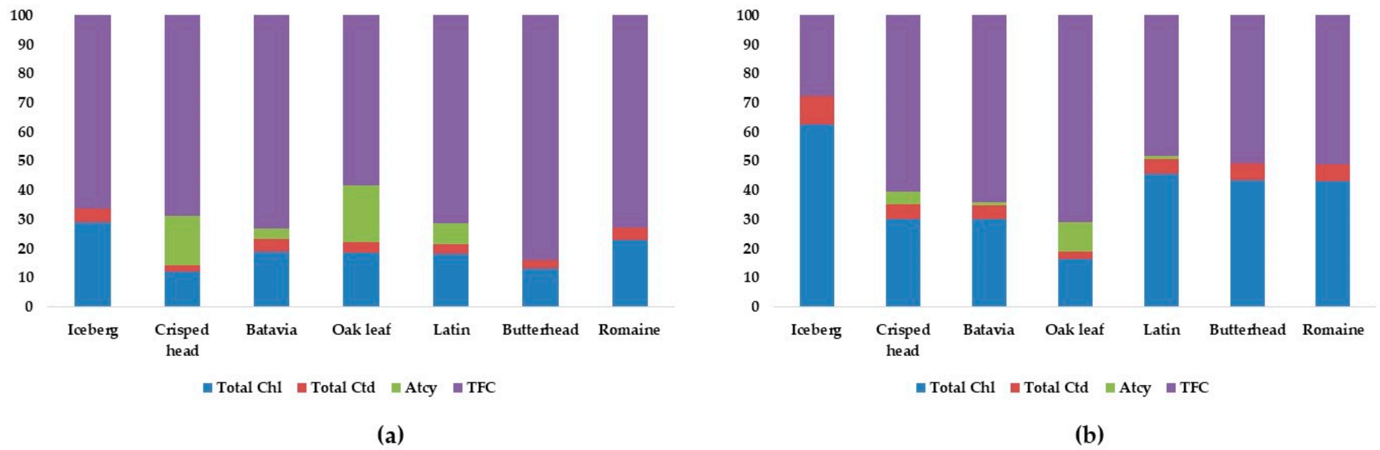

Figure 1. Relative composition of total chlorophylls (Chl), carotenoids (Total Ctd), anthocyanins (Atcy) and total phenolic compounds (TFC) of 7 types of lettuces in: (**a**) field production; (**b**) greenhouse production.

The antioxidant activity of the lettuce under both production systems assessed by the technique using free radical DPPH is shown in Figure 2. It was found that between the production systems studied, there were significant differences ($p < 0.05$). Butterhead, Latin and Iceberg types in the greenhouse system had greater antioxidant capacity.

Principal component analysis (PCA) was used to explore the variation and identify potential patterns within the dataset and look into the relationships between the concentrations of bioactive compounds in lettuce cultivars. The result of principal component analysis showed that the two principal components (PC1 and PC2), explained 60.42% of the total variance (Figure S1). As it is shown in Figure S1, the lettuce cultivars were divided in two groups on the principal component analysis map according to production system. Lettuce cultivars obtained under field production were associated with higher concentrations of total phenolic content, anthocyanins content and fresh plant weight. Lettuce cultivars under greenhouse production were grouped closely due to their higher contents of chlorophylls, carotenoids content and antioxidant activity.

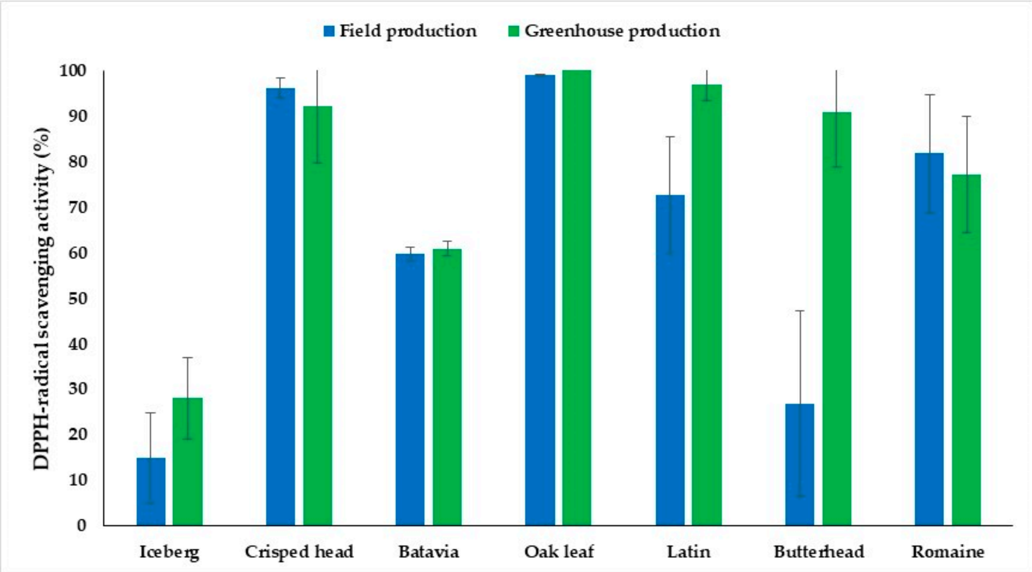

Figure 2. Antioxidant activity expressed as 2.2-diphenyl-1-picril-Hidrazil -radical scavenging activity (%) of 7 types of lettuces in two cropping systems: field and greenhouse production.

4. Conclusions

The present study provides evidence that there is great variability for bioactive compound content, antioxidant activity and growth behavior among lettuce genotypes from different morphological types produced under field and greenhouse systems. It is crucial to know the best agronomic methods and cultivars to maximize the contents of bioactive compounds with health-promoting properties.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Stem diameter, height, fresh weight plant and color parameters of lettuces genotypes in two cropping systems. Table S2: Chlorophylls, total carotenoids, total anthocyanins, and total phenolic compounds of 7 types of lettuces in two cropping systems. Table S3: Chlorophylls, total carotenoids, total anthocyanins, and total phenolic compounds of lettuces genotypes in two cropping systems. Figure S1: Principal component analysis of lettuce genotypes in field and greenhouse production.

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