

## Eco-physiological responses and biochemical characterization of different accessions of *Corchorus olitorius* (L.)

Andrea Giro<sup>1\*</sup>, Antonio Ferrante<sup>2</sup>

<sup>1</sup> Department of Agronomy, Food, Natural Resources, Animals and Environment  
University of Padua, Viale dell'Università 16, 35020 Legnaro (PD), Italy

<sup>2</sup> Department of Agriculture and Environmental Sciences – Production, Landscape, Agroenergy  
Università degli Studi di Milano, Via Celoria 2, 20133 Milano (MI), Italy

### ABSTRACT

*Corchorus olitorius* (L.) is one of the main crops for fibre production, cultivated in tropical and subtropical environments, from Africa to Japan, across the Middle East and India. In developing countries, the leaves are used as a medicine and also as food. In this study, we evaluated the physiological responses of nine accessions of *C. olitorius* in order to nominate the most suitable one for introduction into southern Europe. African and Asian accessions obtained from the Leibniz Institute IPK gene bank were analysed for their photosynthetic variables and quality traits during cultivation. Accessions from Africa, in particular those from Egypt and Libya, showed higher intrinsic water-use efficiency. However, at high temperatures, the Japanese accession also showed a high level of water-use efficiency, as evidenced by the high carbon fixation rate. Chinese and Indian accessions showed a reduction in growth performance, although a high concentration of antioxidants in the leaves and biomass accumulation have been reported in those accessions. *C. olitorius* demonstrated an intrinsic attitude to adaptation; according to their subtropical origin, all of the plants grew at sub-optimal status (Fv/Fm). These data, however, do not seem to compromise the potential of these plants as a crop for growing in southern Europe. Moreover, their introduction can be based solely on their biochemical quality traits.

Key words: African vegetable, antioxidants, biomass, jute, gas exchange, water-use efficiency

### Abbreviations:

DI<sub>0</sub>/CS – Dissipation energy per cross-section, DI<sub>0</sub>/RC – Dissipation energy for reaction centre, ABS/RC – absorbed flux energy per reaction centre, ET<sub>0</sub>/CS – electron transport flux per cross-section, TR<sub>0</sub>/RC – trapped energy flux per reaction centre, Fv/Fm – maximum quantum yield of PS II photochemistry, PI – performance index

### INTRODUCTION

Jute (*Corchorus olitorius* L., n = 24) cultivation has a great economic value in terms of worldwide fibre use, but the plant is also used as a leafy vegetable in Africa and Asia. Moreover, several communities, particularly in Asia, use *C. olitorius*

as a nutraceutical plant (Resources Council, Science and Technology Agency, Japan, 2000). There are several studies which confirm that *C. olitorius* originates from Africa. In particular, Kundu et al. (2013) identified West Africa as the centre of origin of *C. olitorius*. However, archeo-botanical evidence

\*Corresponding author.  
e-mail: andrea.giro@unipd.it (A. Giro).

showed that *C. olitorius*, like *C. capsicaris* (L.), was domesticated in the Indo-Burma region (Wright et al., 2012). Despite *C. olitorius*'s economic benefit to many countries, including India, Bangladesh and China, limited knowledge of the plant's morphological and physiological aspects has halted its genetic improvement (Kundu et al., 2013). Moreover, the lack of a relationship between geographical origin and molecular classification could be due to genetic exchanges among species near the border, thus reducing differences among accessions. Moreover, a correlation between genome size and phenological traits was reported by Benor et al. (2011), although the same study also confirmed the difficulties with finding a significant relationship between genome size and geographical origins. This morphological plasticity allows *C. olitorius* to adapt to a large number of environmental conditions, from Africa to Japan. Hence, transpiration and gas exchange can be essential factors in selecting the best varieties in different geographical areas. These variables are linked to physiological conditions belonging to morphology and genetic traits, although it is interesting to see that a positive correlation between genome size and the microclimatic level has already been discovered among Ethiopian accessions (Benor et al., 2011). Furthermore, this correlation is supported by several studies: Bancheva and Greilhuber (2006), Smarda and Bures (2006), Achigan-Dako et al. (2008). This study can represent a step forward in understanding the plasticity of *C. olitorius* through analysing several geographical accessions worldwide. Physiological, morphological and biochemical variables were evaluated to establish differences and correlations in order to introduce *C. olitorius* as a novel crop into Europe.

Moreover, *C. olitorius* will be introduced into Europe as a functional food thanks to the polyphenols that are prevalent in its leaves (Giro and Ferrante, 2016). The accumulation of these important compounds inside the leaves of a plant is linked to several biotic and abiotic stresses; in particular, to photorespiration and photo-inhibition. Hence, different combinations of polyphenols characterize each vegetable in response to stresses and the environment (Parida et al., 2002). However, different accessions have their own growing behaviour and distinct genetic attitudes that influence biosynthesis and accumulation of secondary compounds at the same time (Favollo et al., 2009). Thus, this study, which was performed in Italy, underlines the variability among *C. olitorius*

accessions from different geographical areas, in particular in terms of quality traits such as the levels of sugars, phenols and carotenoids.

## MATERIAL AND METHODS

### *Plant material and growing system*

Accessions were acquired from the Leibniz Institute's IPK Genebank, using their supply of *C. olitorius* seeds (Tab. 1). The seeds were sown directly into 10 L (0.28 × 0.28 m) plastic conical boxes, using peat as a substrate (class 3), pH 6, EC 0.3 dS m<sup>-1</sup>, density 170 kg m<sup>-3</sup>. Four biological

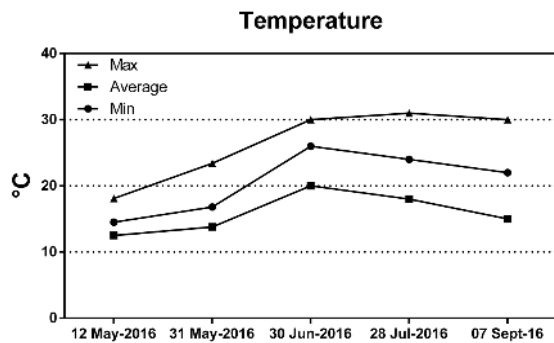
**Table 1.** Origin of the accessions of *Corchorus olitorius* (L.) delivered by IPK (Gene Bank of the Leibniz Institute)

Accession	Region
C1	India
C2	China
C3	Libya
C4	Libya
C5	Libya
C6	Libya
C7	Tunisia
C10	Egypt
C11	Japan

replicates of each accession were cultivated in a protected greenhouse during germination (March and April 2016), after which time the plants, bearing four to six leaves, were moved outside for eco-physiological measurements. Temperature (Fig. 1) and light intensity (Fig. 2) were measured during the cultivation cycle. An automatic irrigation system was used for water supply in order to guarantee the optimum water content in the substrate. Fertilizers were applied to provide macronutrients: 6.5 N, 3 P, and 15 K (g per plant). Mature leaves were collected for biochemical analyses in June 2016.

### *Determination of chlorophylls and carotenoids*

Chlorophyll and carotenoid pigments were extracted from fresh tissues of a mature leaf using 99.9% methanol as solvent. Samples were kept in a dark room at 4°C for 24 hours. Quantitative chlorophyll determinations were carried out immediately after extraction. Absorbance readings were taken at 665.2 and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophyll and carotenoid concentrations were calculated by Lichtenthaler's formula (1987).

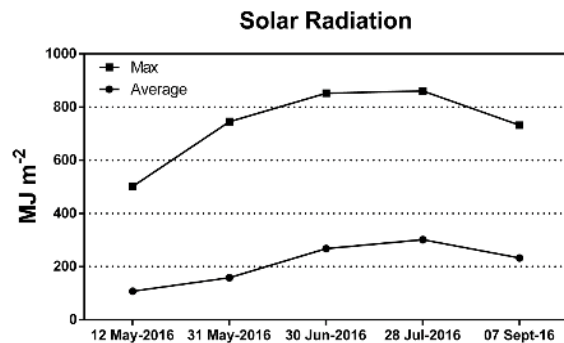


**Figure 1.** Temperature (°C) during cultivation cycle from May 2016 to September 2016 at Università degli Studi di Milano

#### *Determination of sucrose, reducing sugars and total sugars*

For the determination of sucrose, samples of about 1 g of leaves were extracted by homogenization in a mortar with 5 mL of distilled water. The samples were centrifuged at  $10,000 \times g$  for 5 min. The sucrose assay was performed by mixing 0.2 mL of crude extract with 0.2 mL of 2 M NaOH and incubated in a water bath at  $100^\circ\text{C}$  for 10 min., then 1.5 mL of hot resorcinol buffer (containing 30% hydrochloric acid, 1.2 mM resorcinol, 4.1 mM thiourea 1.5 M acetic acid) was added to the samples and incubated in a water bath at  $80^\circ\text{C}$  for another 10 min. After cooling to room temperature, the optical density was determined spectrophotometrically at 500 nm, using a sucrose standard curve (0, 0.5, 1, 1.5 and 2 mM) (Rorem et al., 1960).

The analysis of reducing sugars was performed by using 0.2 mL of crude extract that was added to 0.2 mL of a solution containing 62.6 mM dinitrosalicylic acid (DNS) and 1.52 M potassium sodium tartrate. The reaction mixture was heated at  $100^\circ\text{C}$  for 5 min., then 1.5 mL of distilled water was added and absorbance readings were taken at 530 nm. The reducing sugars were expressed as glucose equivalents using a glucose standard curve (0, 1, 2, 3 and 4 mM) (Miller, 1959). The total sugars in leaves were determined spectrophotometrically following the anthrone method (Yemm and Willis, 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared by dissolving anthrone in ice-cold 95%  $\text{H}_2\text{SO}_4$ . The reagent was left to stand for 30-40 min. before use and 0.5 mL of leaf extract was placed on top of 2.5 mL of anthrone reagent incubated in ice for 5 min. and then vortexed vigorously. The reactants were heated to  $95^\circ\text{C}$  for 10 min. and left to cool in ice. Readings were taken at 620 nm. The calibration curve was constructed using a glucose solution.



**Figure 2.** Solar radiation ( $\text{MJ m}^{-2}$ ) during cultivation cycle from May 2016 to September 2016 at Università degli Studi di Milano

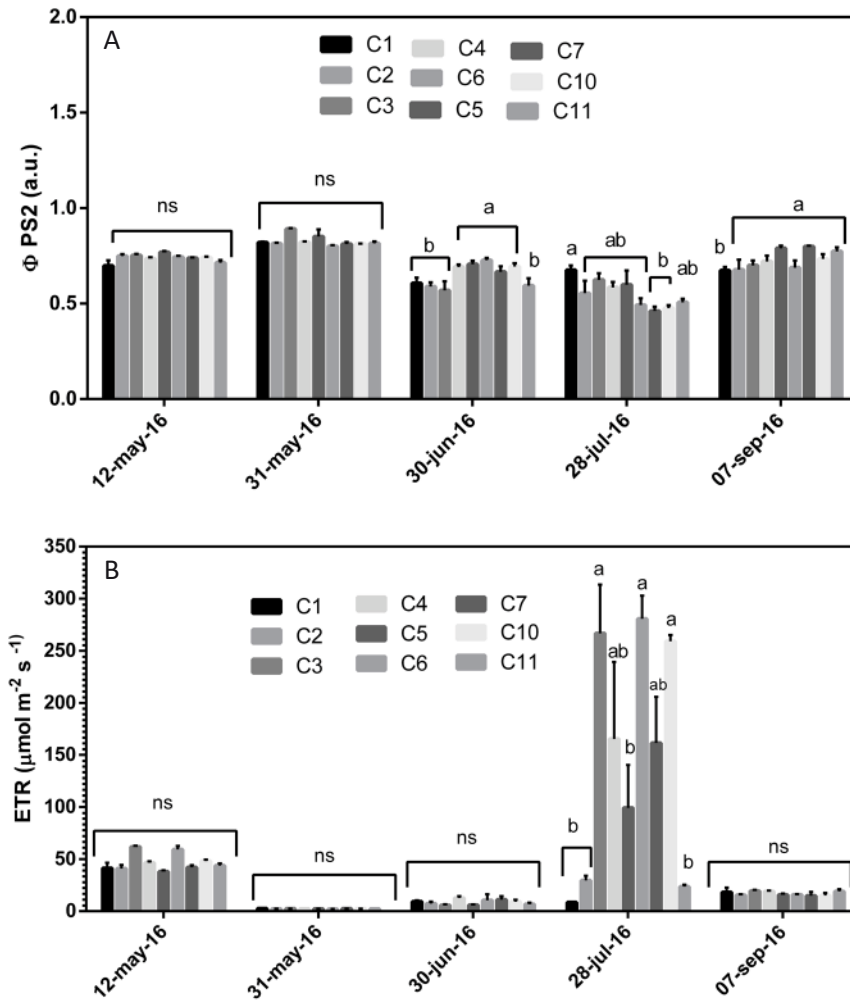
#### *Determination of phenolic compounds and anthocyanins*

Phenols were determined spectrophotometrically in fresh samples following two different approaches: (i) direct measurement of the absorbance of a methanolic extract at 320 nm (phenolic index) and (ii) using the Folin-Ciocalteu method (Ke and Saltveit, 1989; Singleton et al., 1999). The phenolic index was expressed as  $\text{ABS}_{320\text{nm}} \text{ g}^{-1} \text{ FW}$ , while the Folin-Ciocalteu method expresses total phenols as  $\mu\text{g g}^{-1} \text{ FW}$  gallic acid equivalents (GAE). For the determination of anthocyanins, samples of frozen tissue (30-50 mg) were ground in a pre-chilled mortar and extracted into methanolic HCl (1%). Samples were then incubated overnight at  $4^\circ\text{C}$  in the dark. The concentration of cyanidin-3-glucoside equivalents was determined spectrophotometrically at 535 nm (Klein and Hagen, 1961).

#### *Physiological variables of leaves*

Gas exchange measurements were performed with a CIRAS-2 portable photosynthesis system (PP Systems, USA). Measurements were taken on young, fully expanded, intact leaves of *C. olitorius* plants every week for three months from mid-May to mid-July 2016. Net  $\text{CO}_2$  assimilation rate, stomatal conductance and transpiration were assessed by setting the  $\text{CO}_2$  concentration of the instrument at  $400 \mu\text{mol mol}^{-1}$ , 50% relative humidity,  $500 \text{ mL min}^{-1}$  airflow and a photon flux density of  $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The instrument was stabilized according to the manufacturer's guidelines.

Chlorophyll *a* fluorescence was randomly measured using selected dark-adapted leaves from mid-May to mid-June and by using leaf-clips and a portable Handy Plant Efficiency Analyser (PEA, Hansatech, UK). Fluorescence variables were calculated automatically:  $F_v/F_m$  or  $(F_m - F_o)/F_m$ . The JIP-test was performed to determine



**Figure 3.** Chlorophyll *a* fluorescence from light-adapted leaves, quantum efficiency of PS2 (A) and electron (B) flux (ETR) in *C. olitorius* accessions. Standard error reported for each mean ( $n = 21$ ). Different letters indicate statistical differences among the accessions at  $p < 0.05$

the following indices: DI0/CS, DI0/RC, ABS/RC, ET0/CS, TR0/RC and PI (Force et al., 2003). A fluorescence monitoring system was used to measure the photosynthetic quantum efficiency of light-adapted leaves and electron transport flux (Fig. 3).

### Statistical analysis

Data were statistically analysed using the one-way ANOVA method and the differences between means were determined using Bonferroni's post-test (Prism 6 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

## RESULTS

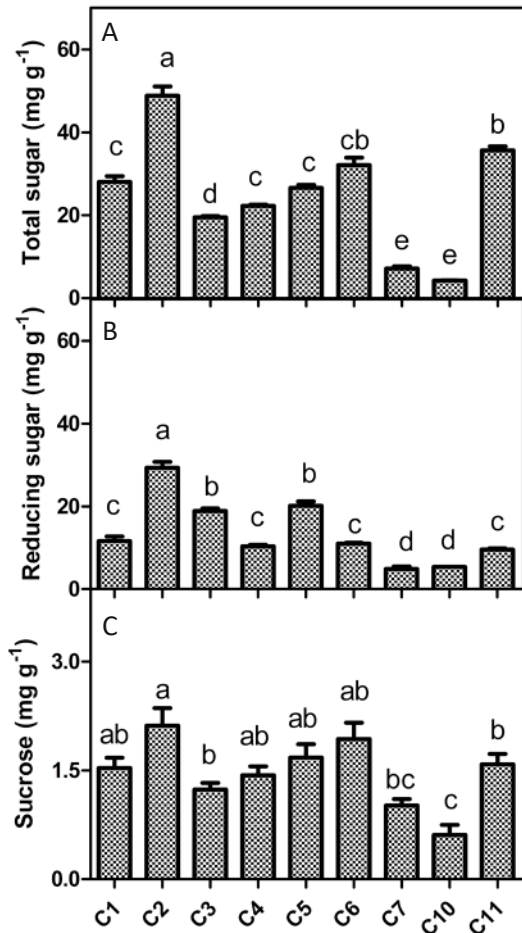
### Sugar content and canopy growth

Among the *C. olitorius* accessions, leaf dry matter (Tab. 2) ranged from 10.45% to 15.13% confirmed significant sugar variation as mainly constituents of carbon skeletons (Fig. 4). However, no significant

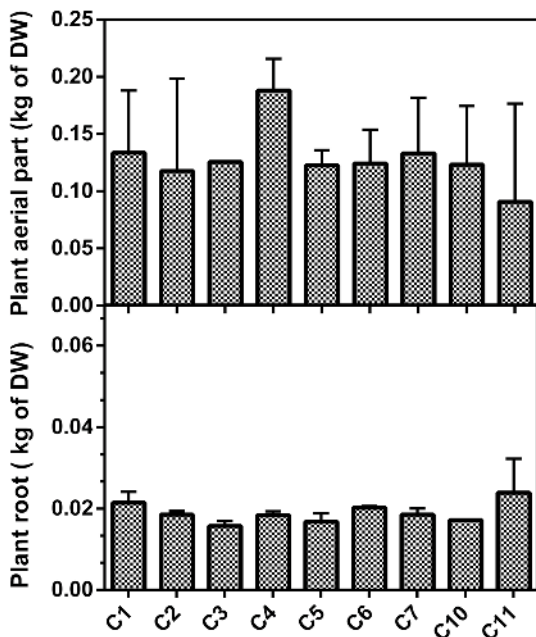
differences in aerial parts DW were recorded after cultivation even though it ranged from 0.09 to 0.18 kg DW (Fig. 5). The level of total sugars varied significantly among the *C. olitorius* accessions. Total sugars ranged from 4.2 to 48 mg g<sup>-1</sup> FW (Fig. 4). Moreover, the variations in sucrose

**Table 2.** Leaf dry matter content of *Corchorus olitorius* accessions

Accessions	Leaf DW (%)
C1	15.13
C2	10.92
C3	11.83
C4	12.33
C5	10.78
C6	10.45
C7	11.78
C10	11.45
C11	12.23



**Figure 4.** Total sugar (A), reducing sugar (B), and sucrose (C) contents (mg g<sup>-1</sup> FW) of *Corchorus olitorius* (L.) plants. Data are means ± standard error (n = 4). \*Explanations: see Fig. 3



**Figure 5.** Seasonal production of *Corchorus olitorius* (L.) in dry weight (DW per plant). Values are means with standard errors (n = 3). \*Explanations: see Fig. 2

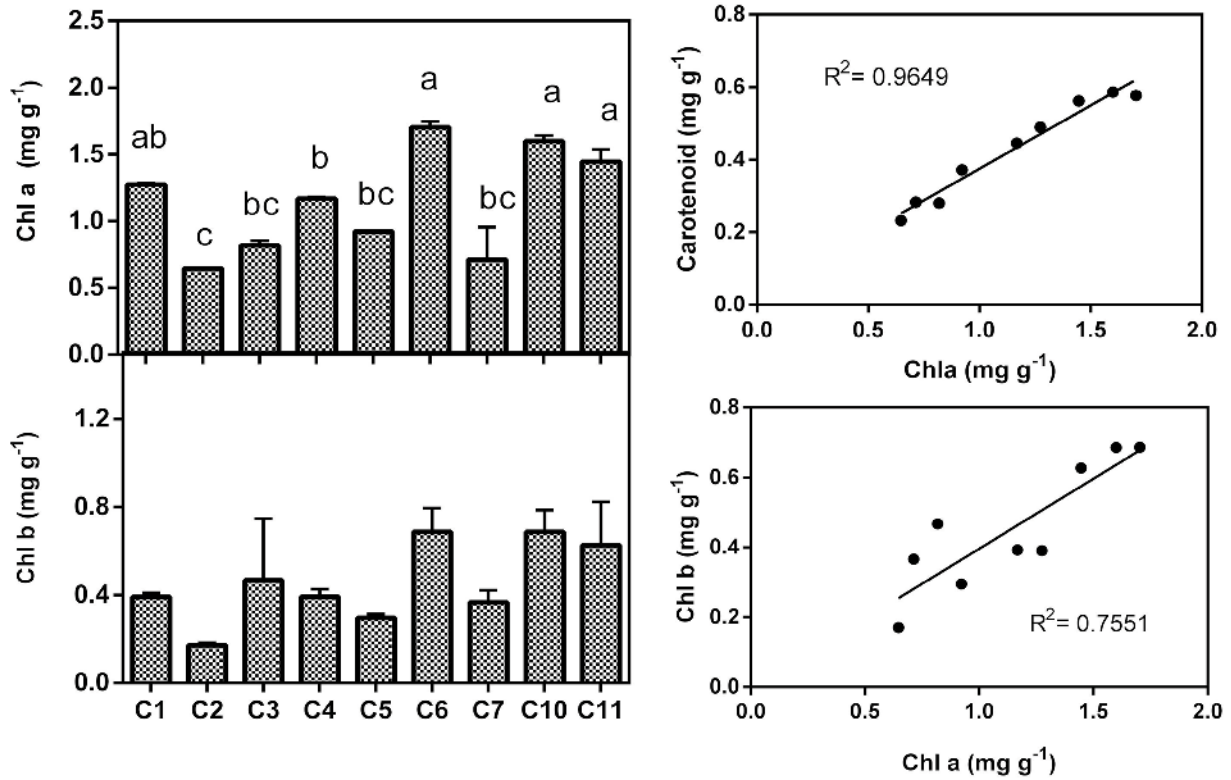
followed the trend of total sugars, demonstrating the differences among *C. olitorius* accessions in the biological activity of carbon fixation and sugar transport inside the leaves. However, the levels of sucrose ranged from 0.6 to 2.1 mg g<sup>-1</sup> FW. The highest values of total sugars were found in C2 leaves (48 mg g<sup>-1</sup> FW), while C6 and C11 had similar values: 32 and 35 mg g<sup>-1</sup> FW, respectively, followed by C1 with 28 mg g<sup>-1</sup>FW. These genotypes showed higher values in comparison with C5 and C4, which had 26 and 22 mg g<sup>-1</sup> FW, respectively. Much lower values were detected in C7 and C10: 7.1 and 4.2 mg g<sup>-1</sup> FW, respectively. The values for sucrose in *C. olitorius* leaves were more clustered than those of total sugars. The highest levels were observed in C2, C6, and C5 with values of 2.1, 1.9 and 1.6 mg g<sup>-1</sup> FW, respectively. Another group comprising C11, C1 and C4 had sucrose values of 1.6, 1.5 and 1.4 mg g<sup>-1</sup> FW, respectively. C3 and C7 had a similar sucrose content of 1.2 and 1.0 mg g<sup>-1</sup> FW, respectively. The lowest amount of sucrose inside *C. olitorius* leaves was found in C10 – 0.6 mg g<sup>-1</sup> FW.

**Chlorophyll content, leaf gas exchange capacity and PSII efficiency**

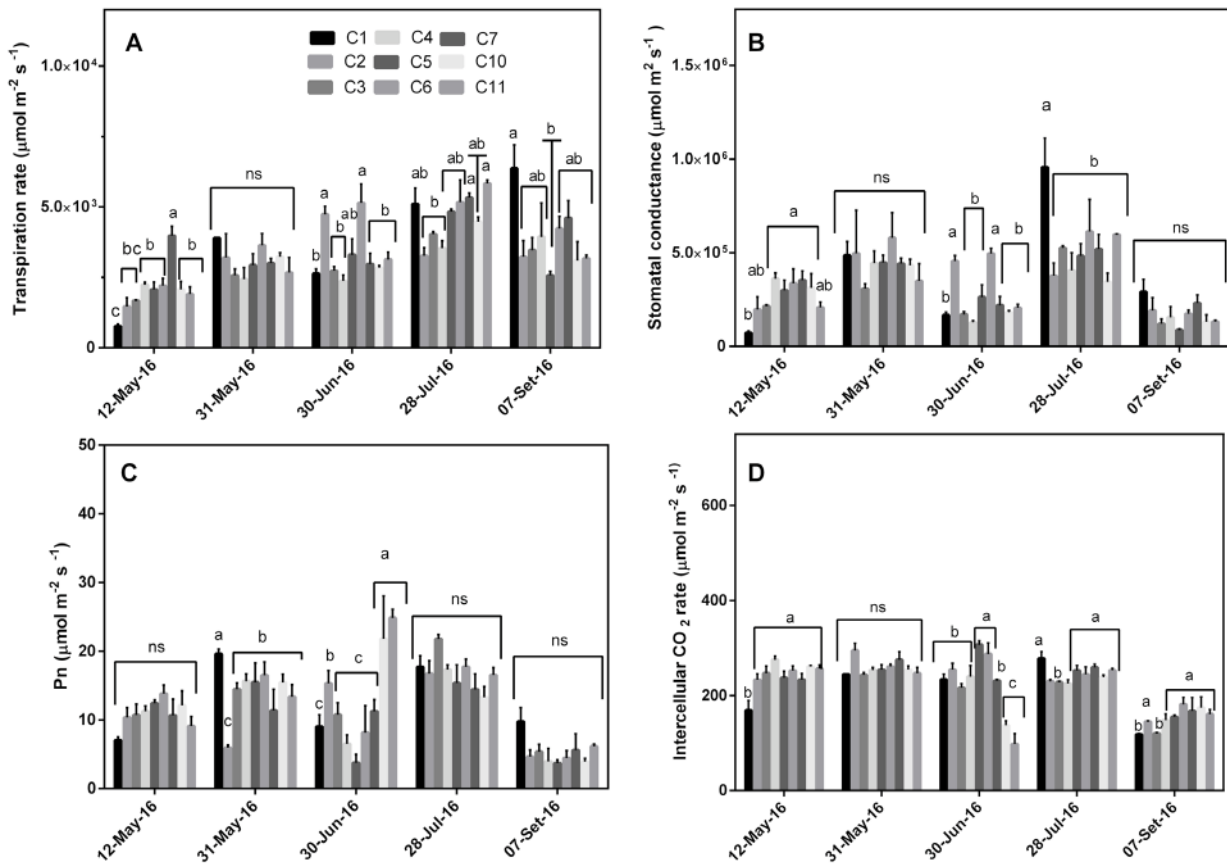
The highest levels of chlorophyll *a* (Chl *a*) were found in C6, C10 and C11. The values ranged from 1.4 to 1.7 mg g<sup>-1</sup> FW. C1 and C4 showed an intermediate chlorophyll content. Chlorophyll *b* (Chl *b*) did not show significant differences between accessions, with values that ranged from 0.17 to 0.68 mg g<sup>-1</sup> FW. Chl *a* in C3, C5, C7 accessions had values of 0.8, 0.9 and 0.7 mg g<sup>-1</sup> FW, respectively. C4 had the second highest amount of Chl *a* – 1.1 mg g<sup>-1</sup> FW; the lowest amount of 0.7 mg g<sup>-1</sup> FW was in C2. Although there were no significant differences in Chl *b* content, values between C2 and C10 were four time higher: C2 (0.17 mg g<sup>-1</sup> FW) to C10 (0.68 mg g<sup>-1</sup> FW). A positive correlation was found between Chl *a* and Chl *b* values, and also between Chl *a* and carotenoids (Fig. 6).

**Leaf gas exchange**

Leaf gas exchange variables, such as stomatal conductance and transpiration, varied significantly among the accessions during cultivation and summertime (Fig. 7D). However, stomatal conductance showed significant differences among the accessions mainly in June and July. Some statistical differences among the accessions in the CO<sub>2</sub> intercellular concentrations were observed during the summer period in June: C10 and C11



**Figure 6.** Chlorophyll *a* and *b* contents of *Corchorus olitorius* plants ( $\text{mg g}^{-1}$  FW). Values are means  $\pm$  standard error ( $n = 4$ ). \*Explanations: see Fig. 3. Direct correlations between chlorophylls and carotenoids are reported

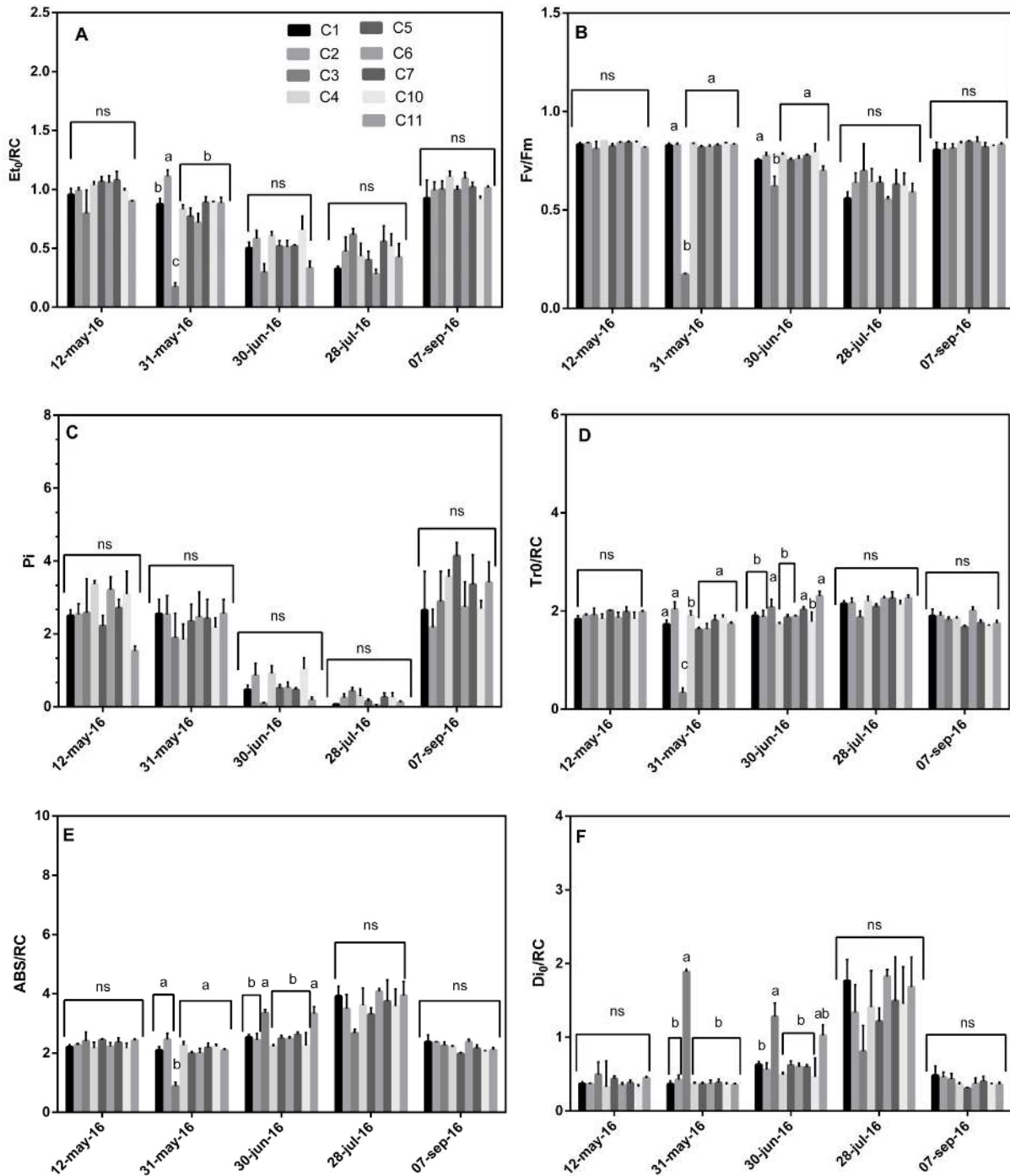


**Figure 7.** Gas exchange analyses during the cultivation of *C. olitorius* accessions. Values are means with standard errors ( $n = 3$ ). \*Explanations: see Fig. 3

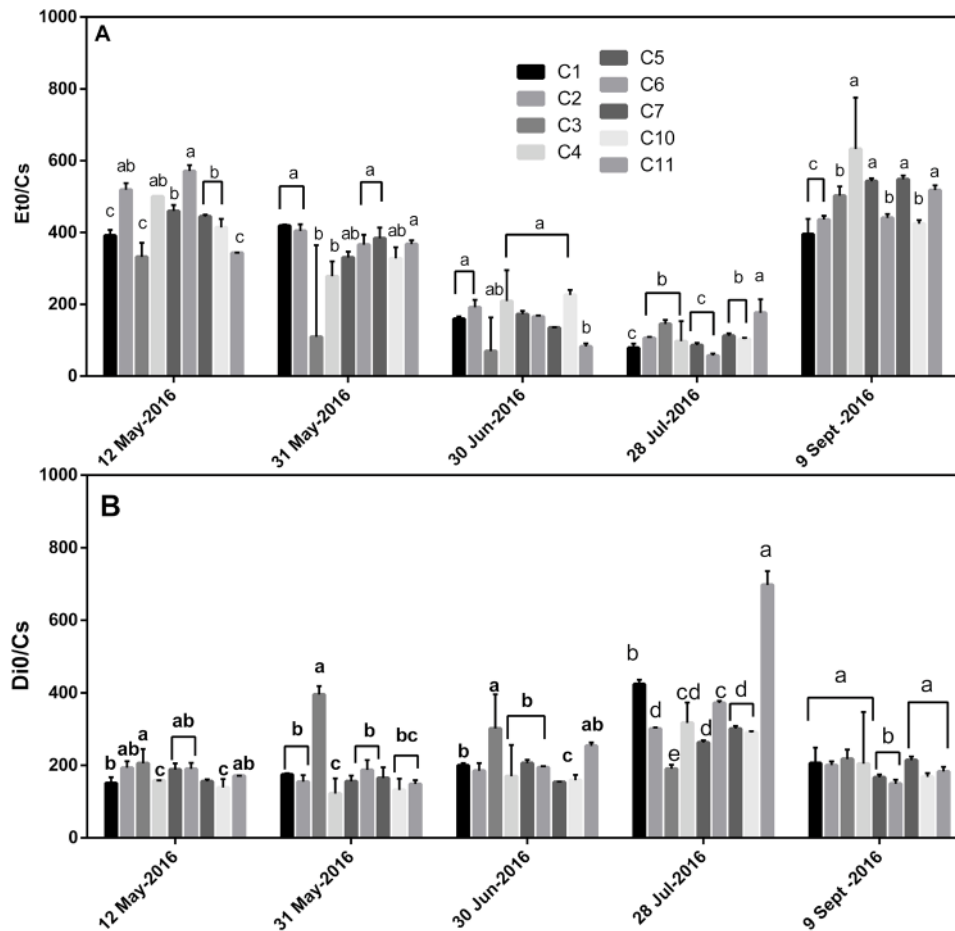
had the lowest levels of stomatal conductance. This was linked to CO<sub>2</sub> assimilation, which could change among the accessions that had the ability to temporarily influence the photosynthetic rate (Pn). On the other hand, in June, C10 and C11 had significantly higher levels of photosynthesis. However, higher average levels of transpiration were found in accessions with lower levels of photosynthesis in June.

**Chlorophyll a fluorescence from dark-adapted leaves**

A few significant differences were found among the accessions in relation to the maximum quantum efficiency of PSII (Fv/Fm), and generally a decreasing trend of efficiency was observed during the cultivation cycle in summertime, with values ranging from 0.69 to 0.77 (Fig. 8). The performance



**Figure 8.** PEA in *C. olitorius* accessions. Values are means with standard errors (n = 8). A – electron transport flux; B – quantum efficiency; C – photosynthetic index; D – trapping; E – absorption; F – dissipated energy flux per reaction centre. \*Explanations: see Fig. 3



**Figure 9.** Quantum yield of electron transport ( $ET_0/CS$ ) (A) and energy dissipation ( $DI_0/CS$ ) (B) in *C. olitorius* accessions. \*Explanations: see Fig. 2

index (PI) did not change significantly among the accessions during cultivation. The  $F_v/F_m$  ratio indicated a general sub-stress condition among the accessions, with all levels below 0.8 ( $F_v/F_m$ ). The performance index (PI) (Fig. 8C) decreased during the summer, with the highest values reached in September: 4.1, 3.4 and 3.5 for C5, C7 and C11, respectively.  $DI_0/RC$  dissipated energy (Fig. 4F) increased with the rise in solar irradiation in the summer (July), with significant differences among the accessions, with the highest values ranging from 0.8 to 1.8.

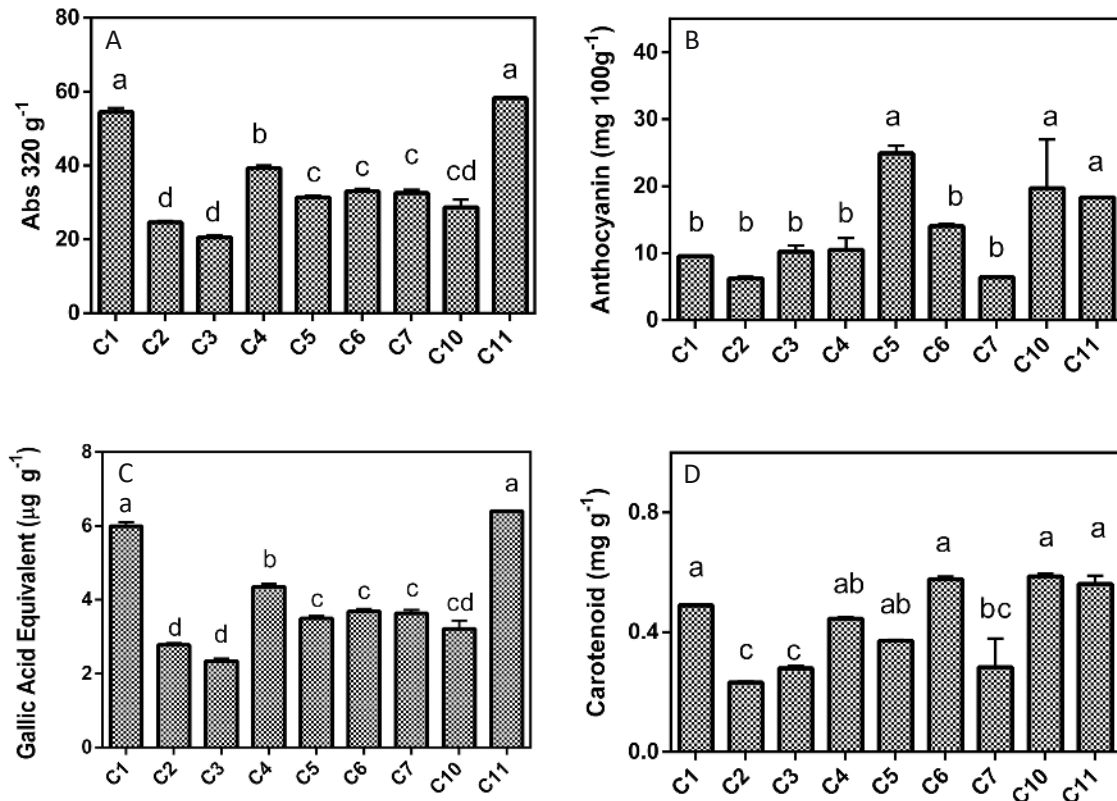
Only on 31<sup>st</sup> May 2016 and 30<sup>th</sup> June 2016 were significant differences found between the C3 accession and the others. In addition to the performance index, the quantum yield of electron transport flux per cross-section ( $ET_0/CS$ , Fig. 9A) also decreased in all accessions during the summer period. A similar trend was found for the average size of antenna and the average absorbed energy flux per reaction centre ( $ABS/RC$ ) (Fig. 8E). In fact, on 31<sup>st</sup> May 2016 and 30<sup>th</sup> June 2016, significant differences were measured among the

accessions which were linked to solar radiation during cultivation, with values of 0.8 and 2.4 for C3 and C2 accessions, respectively. On 31<sup>st</sup> May 2016, the C3 accession showed expected behaviour – a lower gas exchange rate (Fig. 8B) as well as lower  $ET_0/RC$ ,  $TR_0/RC$ ,  $F_v/F_m$ , and  $ABS/RC$ .

#### *Chlorophyll a fluorescence from light-adapted leaves*

Electron transport flux measurements confirmed the previous analysis of ETR with a decreasing trend during the summer period, with values changing from a range of 0.7–0.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to a range of 0.4–0.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . However, the electron transport flux of C3, C7 and C10 increased on 28<sup>th</sup> July 2016, ranging from 0.47 to 0.62 to 0.47  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , in comparison with that of C1, C2 and C11. Moreover, the photosynthetic quantum efficiency percentage showed the main differences among the accessions during the summer measurements of 30<sup>th</sup> June 2016 and 28<sup>th</sup> July 2016 (Fig. 3), confirming the values of the performance index (PI) that also decreased during the summer.





**Figure 10.** Phenolic (A) index (Abs<sub>320nm</sub> g<sup>-1</sup>), anthocyanins (B) expressed as cyanidin-3-glucoside, total polyphenols (C) expressed as gallic acid equivalents, carotenoids in leaves of *C. olitorius* accessions expressed on fresh weight (FW) basis. Values are means with standard errors (n = 4). \*Explanations: see Fig. 3

**Bioactive compounds: polyphenols, total anthocyanins and total carotenoids**

The phenolic index revealed significant differences in total polyphenols among the accessions of *C. olitorius*. The highest values of polyphenols were measured in accession C1 and C11 (India and Japan), with absorbance values of 54.2 and 58.31 ABS<sub>320nm</sub> g<sup>-1</sup> FW, respectively. The lowest values were measured in the varieties C2 and C3 (China and Libya), with values of 24.1 and 20.1 ABS<sub>320nm</sub> g<sup>-1</sup> FW, respectively. Accessions C5, C6, C7, and C10 had values of 31.3, 33.0, 32.5, and 28.1 ABS<sub>320nm</sub> g<sup>-1</sup> FW, respectively. Moreover, the same trends were found for the phenolic content (GAE) in the leaves. The accessions C1 and C11 showed the highest amounts of phenols, with respective values of 5.99 and 6.40 mg g<sup>-1</sup> FW, while C4 showed 4.35 mg g<sup>-1</sup> FW. The accessions C5, C6 and C7 showed values of 3.50, 3.68, 3.63 mg g<sup>-1</sup> FW, respectively. The C10 accession had an average content of 3.21 mg g<sup>-1</sup> FW, similar to what was found in the C5, C6 and C7 group, while the lowest amounts of 2.77 and 2.34 mg g<sup>-1</sup> FW were found in C2 and C3, respectively (Fig. 10).

The amounts of anthocyanins varied among the accessions; however, the anthocyanin content was clustered in only two main groups. High anthocyanin content was measured in C5, C10 and C11, with values ranging from 18.3 to 24.9 mg 100 g<sup>-1</sup> FW; accessions C1, C3, C4, C7 and C6 ranged from 6.3 to 14.6, with no significant differences among them. The lowest levels were detected in C2 leaves – 6.2 mg 100 g<sup>-1</sup> FW. Moreover, carotenoid and chlorophyll levels in *C. olitorius* leaves changed significantly between different accessions.

In fact, the amount of carotenoids in *C. olitorius* leaves changed in correlation with chlorophyll according to their physiological action of supplementary pigments. Despite this correlation, significant differences in carotenoid content among the accessions were less pronounced than those for chlorophyll. The carotenoid level in C1 leaves was 0.48 mg g<sup>-1</sup> FW, i.e. similar to those of C6, C10 and C11, which ranged from 0.56 to 0.58 mg g<sup>-1</sup> FW. In the other accessions C2, C3, C4, C5 and C7, the carotenoid content was: 0.23, 0.27, 0.44, 0.37 and 0.28 mg g<sup>-1</sup> FW, respectively. The C2 accession contained the lowest amounts of carotenoids and anthocyanins.

**Table 3.** Accession-specific mean values of photosynthetic water-use efficiency pWUE ( $P_n/E$ ) and intrinsic water-use efficiency iWUE ( $P_n/g_s$ ). Values are means with standard errors (n = 25). \*Explanations: see Fig. 3

Accessions	pWUE			iWUE		
	Mean	Error	Differences	Mean	Error	Differences
C1	4.52	1.29	ns	0.049	0.012	ns
C2	3.74	1.05	ns	0.033	0.006	ns
C3	4.58	0.86	ns	0.049	0.003	ns
C4	4.02	0.96	ns	0.037	0.004	ns
C5	3.40	0.98	ns	0.033	0.005	ns
C6	3.37	0.96	ns	0.028	0.004	ns
C7	2.83	0.47	ns	0.032	0.004	ns
C10	4.51	1.12	ns	0.052	0.015	ns
C11	4.50	1.03	ns	0.056	0.015	ns

**Table 4.** Total biomass of *Corchorus olitorius* accessions expressed in kg of dry matter. Values are means with standard errors (n = 3). \*Explanations: see Fig. 3

Accessions	Mean	Error	Differences
C1	0.155	0.081	b
C2	0.136	0.025	c
C3	0.127	ns	c
C4	0.208	0.028	a
C5	0.141	0.015	b
C6	0.141	0.030	b
C7	0.157	0.057	b
C10	0.142	0.006	b
C11	0.106	0.010	c

### Water-use efficiency

Water-use efficiency, as well as sugar accumulation in the leaves (Fig. 4), showed differences among the accessions from season to season. Significant variations among the accessions occurred during the growing season, particularly during the summertime. However, considering the mean for the entire period, there were no significant differences between the values for both parameters: photosynthetic water-use efficiency (pWUE) and intrinsic water-use efficiency (iWUE), as shown in Table 3. In contrast, total biomass per plant (aerial parts + roots) in Figure 5 showed some statistical differences among the accessions (Tab. 4). In particular, C4 had the highest biomass production during the production cycle (Tab. 4). Considering the general trend of *C. olitorius* accessions during the growing season, there was a clear progressive reduction in pWUE from May 2016 to September 2016, with a 76% reduction in water-use efficiency.

### DISCUSSION

The tested accessions of *C. olitorius* showed significantly different variations in several compounds such as sugars, phenols, anthocyanins, chlorophylls, and carotenoids. Accessions C1 and C11 contained significantly higher amounts of antioxidant compounds compared to the other accessions. The accumulation of these antioxidant compounds occurs in response to stresses. In particular, phenols are influenced by solar irradiation, which increases the accumulation of these compounds in leaf tissues (Larsson et al., 1986). The anthocyanin content found in *C. olitorius* was similar or slightly lower than in other coloured leafy vegetables such as radicchio (*Cichorium intybus* var. *foliosum*) and red cabbage (*Brassica oleracea* convar. *capitata* var. *rubra*) – 36.9 and 71 mg 100 g<sup>-1</sup> FW, respectively (Kaulamnn et al., 2014). Moreover, *C. olitorius* showed a phenolic content similar to that of other vegetables such as parsley (1.8 mg g<sup>-1</sup> FW), lettuce (0.7 mg g<sup>-1</sup> FW), radish (1.8 mg g<sup>-1</sup> FW), and green bean (0.35 mg

$\text{g}^{-1}$  FW) (Marinova et al., 2005; Marin et al., 2015). However, high antioxidant activity of the plant has already been demonstrated by Yan et al. (2013), as well as its health-promoting properties (Islam, 2013).

Chlorophyll is an important leaf pigment involved in light-use efficiency (Houborg et al., 2011). The C6, C10, C11 and C1 accessions had a statistically higher Chl *a* content. Chl *a* is considered an essential pigment for photo-absorption in leaf thylakoids (Li et al., 2000). However, plants have the ability to regulate their biosynthesis and act in order to reduce photo-oxidation and light burn damage. Significant reductions in trends among some species between ABS/RC and chlorophyll levels are reported, such as C1 during the cultivation cycle. Moreover, our analysis showed that the amounts of Chl *a* and Chl *b* influenced the ABS/RC ratio among the varieties such as C2. Indeed, the concentration of the pigments influenced the absorption capacity of the leaves. Furthermore, energy absorption by thylacoid receptors also influenced the carotenoid content. Carotenoids are secondary compounds which are able to protect leaves against photoreception, and dissipate absorbed energy (Young, 1991). It is important to consider photosynthesis as a biochemical process, which is strongly correlated with plant plasticity and different environmental conditions.

Sugar contents varied considerably among the accessions of *C. olerius*, which was probably linked to different metabolisms and sugar translocation. In fact, in the plants of C1, C2, C3, C4 and C5, the levels of sugars were higher than expected, considering the measurements of carbon fixation rates, which were in line with those of other accessions, such as C11 or C10. However, *Corchorus* species are C3 plants, which means that they lose about 30-50% of their photosynthetic products during photorespiration, as suggested by a sub-optimal Fv/Fm ratio (Fig. 7). It is likely that C1, C2, C3, C4 and C5 plants had a lower photorespiration rate that could increase triose sugar deposits in leaves. In fact, triose sugars could be stored in chloroplasts as primary amide (Huber, 1982). This data is supported by the differences among the accessions of *C. olerius* in the total amounts of sugar in leaves. Sucrose, for example, does not generally show high significant variations among accessions. Sucrose is, however, significantly higher in accessions with lower respiration rates, such as C2. This aspect can be explained by a faster metabolism, which consequently transports triose sugars in the form of sucrose to be exported in

vascular tissues, as suggested by the yield of dry matter (Kaiser and Heber, 1984; Ward et al., 1997).

Physiological activity of photosystem II is a key factor in the leaf during cultivation (Long et al., 1994). High solar irradiation causes biochemical changes in photosynthesis patterns, which could inhibit electron transport flux ( $\text{ET}_0/\text{RC}$ ), causing a reduction in carbon fixation by the saturation of the system (Fig. 7 and 8). Moreover, stomatal conductance is an important variable for understanding water potential per photosynthetic rate ( $P_n$ ) to estimate the photosynthetic water-use efficiency (pWUE) (Ball et al., 1987).

Generally, during photo-inhibition,  $\text{DI}_0/\text{CS}$  increases and  $\text{ET}_0/\text{CS}$  decreases, as was the case during the measurements taken in the summer (Fig. 3). Accession C3 previously showed an increase in  $\text{ET}_0/\text{RC}$  in comparison with the others. A similar trend is suggested by the C3, C7 and C10 accessions that increased electron transport flux on July 28<sup>th</sup>, linked at the same time to the reduction in quantum efficiency. This is probably a consequence of the dissipation of energy ( $\text{DI}_0/\text{RC}$ ), as suggested by accession C10, which contained a higher amount of carotenoids.

The stresses caused by light could influence photorespiration, while photosynthetic stresses and dissipation, particularly during the summer, can produce  $\text{O}_2$ , which increases photorespiration and the consumption of sugars (Cuée et al., 2006; Voss et al., 2013). In our case, there were significant differences during the summer in the photosynthetic quantum efficiency status of photosystem II (Fig. 3) among the accessions in light-adapted conditions. Taking into consideration the values of  $\text{DI}_0/\text{CS}$  and  $\text{ET}_0/\text{CS}$  of the accessions, the sugar levels in the leaves could suggest different behaviour in sugar storage and the use of starch. Although sucrose and reducing sugars were at different levels in the accessions of *C. olerius*, neither variable was influenced by the photosynthetic rate, since sucrose is a transport sugar and reducing sugars are a metabolic substrate in cytosol (Lemoine et al., 2013). Moreover, variations in the gas exchange rate suggested that there was a relationship between photosynthetic rate and stomatal conductance. This combination of factors might generate a higher use of sugars in leaf tissues, probably causing respiration, as suggested by the leaves of C7 and C10. Moreover, the data suggested that plants of C10, C11, C7 and C6 had a high carbon fixation rate, but generally less efficient use of sugars in comparison with the metabolism of C1, C2, C3

and C4. Meanwhile, the data in Figure 8 suggested that the accessions from Libya had a lower photo-inhibition response during the summer period, showing good adaptation to high temperatures and solar radiation. However, at the same time, during the summer period (June-July), the water-use efficiency of the Libyan accession (C4) decreased dramatically in comparison with that of the other accessions. This could be explained as a form of adaptation to North African temperatures, thanks to a higher water transpiration rate. In fact, during June and July, measurements of C4's photosynthetic quantum efficiency were not influenced by the high temperature. Moreover, C4 turned out to be one of the better accessions, considering its photosynthetic quantum efficiency (Fig. 3).

The increase in temperature could produce a vapour pressure deficit (VPD) that had a direct negative effect on WUE (Kaminski et al., 2015). In fact, VPD influenced the leaf, increasing the transpiration rate. Thus, the intrinsic water-use efficiency value underwent a lower reduction compared to pWUE during the test, with a 26% decrease in mean from May 12<sup>th</sup> to September 7<sup>th</sup>. According to the mean values of pWUE of crops such as potatoes (Kaminski et al., 2014), *C. olitorius*, in many cases, had values similar to those of C1 (India) or C3 (Libya) accessions. Moreover, plant biomass was generally allocated to the upper part of the plant and not to the roots. In general, the C3 accession allocated, like wheat, more than 30% of its biomass to the roots (Kalapos et al., 1996; Spiesman et al., 2018). However, the *C. olitorius* accessions generally had a lower root biomass percentage, with values ranging from 10 to 20%, which are similar to those of a plant with the physiology of C4 (Kalapos et al., 1996).

Water limitation and water-use efficiency will be a major issue in the future of agriculture due to the increase in global warming. The increase in temperature will raise the water vapour concentration difference between the air and the leaves, although relative humidity in the atmosphere is foreseen to be constant on a global scale (Willet et al., 2007). According to this general behaviour, accessions C10 and C11 showed high intrinsic WUE at high temperatures.

Unfortunately, all of the accessions had values of photosynthetic quantum efficiency of less than 1% during the entire growing cycle. Generally, typical crop plant performances are in the range of 1-2% photosynthetic efficiency. Despite the mean values of electron transport flux being under 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$

in all the data collected in the July measurement of the North African accessions C3, C4, C5, C10, C7 and C6 have mean values ranging from 100 to 280  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Considering the high values of temperature and light during July in a Mediterranean environment, the increment of electron transport flux could be an adapting response to light stress linked to the native North African origin of these plants. However, this increase in electron transport flux does not generate a higher photosynthetic rate, but it could be a response of energy dissipation (Ruban and Horton, 1995).

## CONCLUSIONS

To understand the difference in growth performance, it is important to find a *C. olitorius* accession that is best adapted to the environmental conditions of northern Italy in order to introduce the best performing and most valuable plant. The results show that the varieties C1 (India) and C11 (Japan) had high concentrations of antioxidant compounds, even though highest sugar levels were found in the Chinese accession (C2). Photosynthetic rate results highlight an interesting light-use efficiency of the C1 accession in comparison with that of C11, although a difference in iWUE has been reported. According to our results, further investigations of the ecology of the African accessions should be conducted, considering the good production level of the Libyan accession C4 thanks to its response to the high-temperature climate. However, considering the general response to environmental conditions, all the accessions tested completed the growing cycle without showing differences in biomass production. Thus, the introduction of *C. olitorius* as a new vegetable can be solely based on the evaluation of its biochemical quality traits allowing selection of accessions according to their antioxidant or sugar content.

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## AUTHOR CONTRIBUTIONS

A.G. – performed plant cultivation, analytical measurements and statistical analysis; A.F. –

designed the experimental plan; A.G. and A.F. – equally contributed to manuscript writing.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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