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Agronomic Performances and Nutritional Value of *C. olitorius* in Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. Author KM designed the study, wrote the sample collection and handling protocol, performed the statistical analyses and drafted the manuscript. Author KZ conceived the theme of the study and read the first draft of the manuscript. Authors SN and SB contributed to the interpretation of the data. Author SZ conducted the trial and collected the data. Authors BKP, SM read and validated the study protocol. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Jute mallow (*C. olitorius*) is a traditional leafy vegetable from Africa and Asia. The richness of its leaves in nutritional elements such a (iron, zinc, potassium and beta carotene) makes it an excellent nutritional supplement for pregnant women and children. It is one of the most important leafy vegetable consumed in Burkina Faso. However, leaf biomass yields remain low and production is still unable to meet the increasing demand. Therefore, the study aims to evaluate the agronomic performances of four morphotypes identified during our previous studies. It will be also question to evaluate their biochemical composition and to study the relationship between agronomic and biochemical traits. Four morphotypes were evaluate agronomical according to Fisher block design using 12 quantitative traits. Fresh leaves of each morphotype were then used for determination of beta-carotene content and mineral element content. The results of the study

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showed great variability in the agronomic performance and biochemical composition of the four morphotypes. The morphotype (SBL1) with green and shiny leaves expressed the best performance in biomass and number of primary branches as well as in nutritional elements such as iron, potassium and beta carotene. This morphotype could be used as a breeding parent in an extension program for the valorization of this leafy vegetable.

Keywords: Biochemical; Bulvanka; Corchorus olitorius; genetic parameters; morphotypes.

1. INTRODUCTION

Leafy vegetables provide an essential part of the nutritional and medicinal needs of populations in most developing countries [1]. They are very rich in mineral elements such as calcium, iron and phosphorus [2] and thus appear as allies in the fight against "hidden hunger", i.e. deficiencies in micronutrients such as vitamin A and mineral including iron, which prevents anemia [1]. Indeed, since 2010, Corchorus olitorius, is recognized as one of the most consumed leafy vegetables in Africa. It is indeed rich in protein, vitamins (A, C, E) and mineral elements [3,4]. In Burkina Faso, it is ranked first or second in terms of consumption according to localities and itis consumed either fresh or dried during all period of the year [5,6]. In addition to its role as a food, the sale of its leaves constitutes a significant source of income for women [5]. Corchorus olitorius is also used as a medicinal plant for the treatment of various diseases [7]. Due to its socio-economic interest, several studies have been undertaken for its valorization and varietal [8,9]. These studies improvement have highlighted the existence of diversity within the species that can be translated into variability in the biochemical composition of the consumed if parts. Indeed. previous biochemical characterization studies [3,10,4]have allowed to highlight the nutritional profile of the leaves, no study has been carried out taking into account the relationship between morphological variability and nutritional value of C. olitorius in Burkina Faso.

In Burkina Faso, four morphotypes of *C. olitorius*were identified during the previous

studies [5] belonging to the varieties *C. olitorius var insicifolius* and *C. olitorius var olitorius*. This study aims at a better exploitation of the nutritional potentialities of *C. olitorius*through the evaluation of agronomic and biochemical composition of the four identified morphotypes of *C. olitorius L.*

2. MATERIALS AND METHODS

2.1 Plant Material

Four morphotypes of *C. olitorius*, namely SAB3 (Fig. 1A) SAR2 (Fig. 1B), KAY1 (Fig. 1C) and SBL1) were used for this study. Each morphotype is made of 3 genotypes. The selection of these genotypes was made taking into account varietal type, stem color, leaf color and brightness. The characteristics of these morphotypes are recorded Table 1. The morphotype SAB3 is characterized by a stem with a red base and green leaves wile SAR2 is characterized by green stem and green leaves. KAY1 is characterized by a green stem and green leaves and SBL1 by a green stem with green and shiny leaves.

2.2 Experimental Site

The agronomic trial was established in Gampèla, with geographic coordinates of 12°15' North latitude and 1°12' West longitude. The site is located in the North Sudanese domain and is characterized by an annual rainfall ranging from 600 to 900 mm [11]. Annual maximum temperatures range from 35 to 40°C and minimum temperatures range from 18 to 19°C.

Table 1. Stem and leaf characteristics of the four C. olitorius morphotyes studied

Morphotypes	Variety	Characteristics						
		Stem color	Leafcolor	shinyLeaf				
SAB3	C. olitorius var. olitorius	Red	Green	Not shiny				
SAR2	C. olitorius var. olitorius	Green	Green	Not shiny				
KAY1	C. olitorius var. incisifolius	Green	Green	Not shiny				
SBL1	C. olitorius var. incisifolius	Green	Green	Shiny				



Fig. 1. Characteristics of the four morphotypes

A- Morphotype SAB3; B- Morphotype SAR2 (C. olitorius var olitorius), C- Morphotype KAY1, D- Morphotype SBL1 (C. olitorius varincisifolius)

2.2.1 Experimental design and cultivation practices

The trial was conducted in a three-repeat Fisher block design between July and October 2016 on a plot previously plowed. Seeds of the four morphotypes were first placed in a nursery after breaking their dormancy by soaking in nearboiling water (100°C). The resulting seedlings were then transplanted to the experimental plot three weeks after nursery. In each replication, each morphotype was transplanted to three lines of seven seedlings each. Thus, the plot had 12 lines of each morphotype. The row spacing and inter-bunch spacing were 0.5 m each and the spacing between replications was 1.5 m.

2.2.2 Agronomic traits study

The agronomic traits measured were 50 % flowering cycle (FLC), plant height (PLH), stem diameter (DTI), primary ramifications number (PRN), leaves number per plant (LNP), Limb length (LIL); Limb width (LIW); petiole length (PEL), fresh weight (FWL) and dry weight (DRW) of leaves and fresh weight of stems (FWS). Leaf dimensions (PEL; LIL; LIW) were indeed measured on three leaves per plant. Except the number of days to 50% flowering determined per

line, the other characters were measured on four randomly selected plant per line.

2.2.3 Biochemical Parameters

The biochemical parameters assessed were water content, β -carotene content and mineral salts. The mineral salts arecopper (Cu), iron (Fe), manganese (Mn), zinc (Zn), total nitrogen (N), total potassium (K), total phosphorus (P) and total magnesium (Mg). These mineral salts were evaluated by atomic absorption spectrometry according to the AOAC 999.11 method [12]. Regarding the β -carotene content, it was evaluated by HPLC method. The water content was determined according to the formula: water content (WC) = (Pf - Ps)/Pf × 100 with, Pf = fresh weight of leaves and Ps = dry weight of leaves.

2.3 Data Analysis

The collected data were entered and processed using Excel 2010. The software XLSTAT pro. 2016 was used for the analysis of variance (ANOVA) and the Newman-Keuls test of separation of means at the 5 % threshold which were performed to compare the agronomic performances and biochemical composition of the studied morphotypes. The same software was also used for the realization of the Pearson correlation matrix to study the links between the agronomic and biochemical variables. In order to determine the contributory part of the genotype in the expression of biochemical and agromorphological traits. some genetic parameters such as H²were calculated from the components of the analysis of variance according to the formulas used by [13] and [14].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Agronomic performances of the morphotypes

The analysis of the average performances of the four morphotypes (Table 2) shows that except the stem diameter (DIS), the fresh weight of stems (FWS) and the fresh weight of leaves (FWL), the other studied characters differentiate the four morphotypes. Thus, the cycle varied from 55 days for the SAB3 morphotype to 88 days for the SBL1 morphotype. Similarly, the SAB3 morphotype recorded the highest dry matter weight and the SBL1 morphotype the lowest dry matter weight. The variables limb length (LIL) and leaf width differentiatesignificantly the morphotypes of the two botanical varieties. The morphotypes belonging to *C. olitorius var olitorius* showed the longest leaves whereas those of the variety *C. olitorius varincisifolius* presented the widest leaves.

3.1.2 Biochemical composition of the four morphotypes studied

The biochemical composition of the leaves of the four morphotypes (Table 3) revealed that only the water content, potassium content and dry matter contentsignificantly difference the four morphotypes at the 5% threshold. The SBL1 morphotype presented the highest value in potassium 3.48 g/100MS against 2.56 g/100MS for the KAY1 morphotype which presented the lowest value. For water content, the SBL1 morphotype also gave the highest content (79.23%), indicating that it has the lowest dry content (20.78%). For the other ters, no significant difference was matter parameters. observed. Nevertheless, the SBL1 morphotype recorded the best values for ß-carotene and iron content.

Considering the varietal factor, no significant difference was observed between the two botanical varieties.

3.1.3 Estimation of genetic parameters of biochemical traits

The results of the estimation of the genetic parameters (Table 4 and 5) show that for all the traits studied, the phenotypic variances are higher than the genotypic variances. Moreover, a low difference of about 3% is observed between the phenotypic (PCV) and genotypic (GCV) coefficients of variation for all morphological traits. Heritability in the broad sense is also high (H²> 50%) for most traits except for stem diameter (49.00) and stem fresh weight (46.058). For biochemical traits, a low difference between the phenotypic (PCV) and genotypic (GCV) coefficient of variation was also noted. This difference is about 3 % for the characters water content, potassium, phosphorus and ß-carotene and higher (3 %) for the other biochemical elements (magnesium, iron, copper, manganese, zinc). A high broad heritability (50 %) was also recorded for these three elements. The genetic gain compared to the average is relatively low for all traits except for potassium (21.90%) and phosphorus (23.38%).

Variables	C. olitorius	var olitorius	C. olitorius	var incisifolius	Моу	C %	F	
					2		Morph.	Var.
	SAB3	SAR2	KAY1	SBL1				
FLC (jrs)	56.33°	66.33 ^b	55.66°	88.00ª	66.58	2.12 ± 1.4	455.32**	1.898 ^{ns}
PEL (cm)	4.86 ^c	6.68 ^b	5.31°	7.51ª	6.09	8.13 ± 0.5	24.376**	0.791 ^{ns}
LIL (cm)	16.07ª	13.76 ^b	10.68 ^d	11.53°	13.01	3.59 ± 0.4	107.43**	37.138***
LIW (cm)	4.58 ^c	5.03°	7.16 ^b	9.41ª	6.54	6.96 ± 0.4	94.74**	43.688***
PLH (cm)	104.44 ^b	118.11ª	100.77 ^b	107.22 ^b	107.6	5.65 ± 6.1	6.03**	2.023 ^{ns}
DIS (cm)	15.28ª	16.03ª	14.52ª	14.90 ^a	15.19	8.41 ± 1.28	1.02 ^{ns}	1.305 ^{ns}
PRN (nbr)	16.22 ^b	20.33ª	17.33 ^{ab}	15.44 ^b	17.33	9.89 ± 1.7	6.26**	1.623 ^{ns}
LNP (nbr)	700.33 ^b	642.33 ^b	933.33ª	525.66 ^b	700.4	14.7 ± 102.6	11.16**	0.267 ^{ns}
FWL (g)	107.81ª	99.19ª	101.31ª	90.13ª	99.61	23.7 ± 23.6	0.38 ^{ns}	0.260 ^{ns}
FWS (g)	706.66 ^a	716.66 ^a	683.33ª	496.66 ^a	650.8	30.69 ± 99.9	1.079 ^{ns}	0.836 ^{ns}
DRW (g)	22.57 ^a	22.30ª	22.30 ^a	20.78 ^b	22.07	2.20 ± 0.48	5.67*	3.746 ^{ns}

Table 2. Agronomic performances of the four morphotypes

*: significant; **: highly significant; ns: not significant; PEL: Petiole length; LIL: Limb length; LIW: Limb width; FLC: 50 % Flowering cycle; PLH: Plant height; DIS: Diameter of the stem; PRN: Primary ramification numbers; FWL: Fresh weight of the leaves; FWS: Fresh weight of the stems;DRW: dry weight of leaves

Table 3. Biochemical performances of *C. olitorius* morphotypes for 100 g of plant material

Variables	C. olitorius va	C. olitorius var insicifolius		ar olitorius	Moy.	C%	F	
	KAY1	SBL1	SAB3	SAR2			Morph.	Var.
TEF (%)	77.73 ^{ab}	79.23 ^a	77.43 ^b	77.70 ^{ab}	77.93	0.62 ± 0.49	5.67*	1.58 ^{ns}
N (g)	0.28 ^a	0.30 ^a	0.34ª	0.30 ^a	0.30	9.88 ± 0.03	1.48 ^{ns}	2.87 ^{ns}
P (g)	0.47 ^a	0.56 ^a	0.61ª	0.45 ^a	0.52	10.09 ± 0.05	3.97 ^{ns}	0.03 ^{ns}
K (g)	2.72 ^b	3.49 ^a	2.78 ^b	2.78 ^b	2.94	5.30 ± 0.16	11.08 [*]	1.81 ^{ns}
Mg (g)	0.13 ^a	0.13 ^a	0.12ª	0.13ª	0.13	11.4 ± 0.01	0.82 ^{ns}	0.70 ^{ns}
Fe (mg)	28.25 ^a	30.65ª	21.20 ^a	19.80 ^a	24.98	36.55 ± 9.13	0.67 ^{ns}	2.82 ^{ns}
Cu (mg)	0.93 ^a	0.57 ^a	2.04ª	0.78 ^a	1.08	84.29 ± 0.91	1.05 ^{ns}	1.04 ^{ns}
Mn (mg)	7.06 ^a	5.74 ^a	8.21ª	7.1 ^a	7.03	20.27 ± 1.42	1.00 ^{ns}	1.71 ^{ns}
Zn (mg)	2.40 ^a	2.64 ^a	2.00ª	2.29 ^a	2.29	23.26 ± 0.53	0.7 ^{ns}	2.15 ^{ns}
ß-carot (µg)	3170.50 ^a	3234.85 ^a	3103.10 ^a	3178.75 ^a	3172	1.24 ± 39.49	3.8 ^{ns}	2.85 ^{ns}

*: significant; **: highly significant; ns: not significant TEF: water content; ß-Car: ß-carotene; Cu: Copper; Fe: Iron; K: Potassium; Mg: Magnesium; Mn: Manganese; N: Nitrogen; P: Phosphorus; Zn: Zinc

Variables	VG	VP	H ²	GCV (%)	PCV (%)	GA	GAx (%)
DIS	0.400	0.816	49.000	4.164	5.948	0.912	6.004
PLH	37.240	55.707	66.850	5.671	6.937	10.278	9.552
LIL	4.812	4.916	97.889	33.512	33.871	4.471	68.302
LIW	5.751	5.860	98.138	18.433	18.607	4.894	37.617
PEL	1.374	1.496	91.795	19.235	20.076	2.313	37.963
NFE	24119	29387	82.074	22.173	24.475	289.834	41.381
FLC	226.657	227.657	99.561	22.612	22.662	30.945	46.479
PRN	3.132	4.601	68.070	7.806	9.461	3.008	13.267
FWL	225.800	279.233	80.864	15.085	16.776	27.836	27.945
FWS	9186.000	19944.333	46.058	14.727	21.700	133.994	20.589

PEL: Petiole length; LIL: Limb length; LIW: Limb width; FND: 50 % Flowering cycle; PLH: Plant height; DIS: Diameter of the stem; PRN: Primary ramification numbers; FWL: Fresh weight of the leaves; FWS: Fresh weight of the stems; VG: Genotypic variance, VP: Phenotypic variance, H²= broad sense heritability; GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation, GA: genetic advance; GA (% of mean) is genetic advance as per cent of the mean

Table 5. Results of genetic parameters for biochemical traits

Variables	VG	VP	H ²	GCV (%)	PCV (%)	GA	GAx (%)
ß-carot	2139	2915.00	73.379	1.458	1.702	81.613	2.573
Cu	0.020	0.435	4.613	13.111	61.041	0.063	5.801
Fe	13.690	41.680	32.845	14.812	25.845	4.368	17.487
K	0.122	0.135	90.972	11.899	12.475	0.687	23.379
Mg	0.000	0.000	18.529	3.472	8.065	0.004	3.078
Mn	0.002	1.017	0.197	0.637	14.352	0.004	0.058
N	0.000	0.001	32.389	4.838	8.500	0.017	5.671
Р	0.004	0.006	74.812	12.292	14.212	0.115	21.902
Zn	0.058	0.142	41.077	10.539	16.444	0.319	13.915
TEF	1.229	1.347	91.176	1.421	1.488	2.180	2.794

N: Nitrogen; P: Phosphorus; K: Potassium; Mg: Magnesium; Fe: Iron; Cu: Copper; Mn: Manganese; Zn: Zinc; ß-Car: ß-carotene; TEF: water conten; VG: Genotypic variance, VP: Phenotypic variance, H²= broad sense heritability; GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation, GA: genetic advance; GA (% of mean) is genetic advance as per cent of the mean

3.1.4 Relationship between morphological and biochemical traits

The study of correlation (Table 6) showed many significant correlations (1%) between agronomic and biochemical traits. β -carotene content was positively correlated with leaf blade width (r = 0.812) and with leaf water content (r = 0.808). Positive correlations were also observed between the number of days 50 % flowering and β -carotene (r = 0.739) and potassium (r = 0.896) content, respectively. Iron content was negatively correlated with stem diameter on the one hand (r = -0.732) and with stem fresh weight (r = -0.71) on the other hand.

3.2 Discussion

Several agronomic and biochemical traits differentiate the four genotypes. In general, *C. olitorius* has a high nutritional value, especially in

ß-carotene and iron, which could contribute to the improvement of the nutritional quality of the population's diet and thus to food security. Similar results have been reported by previous studies [15], which suggested the use of C. olitorius to meet the iron and ß-carotene requirements of children and pregnant women. The ß-carotene value (3172 µg/100 g MF) obtained in this study is much higher than the 1000 µg/100 g MF obtained by [4]in Cote d'Ivoire. The mean iron value of 24.98 mg/g dry matter is higher than that obtained by [10], on samples from Cameroon (6.05 mg/g) and [16], on samples from South Africa (22.8 mg/g). These differences could be dueto the genotypes used.According to [17], variation in chemical composition is primarily related to genotypes. In addition, environmental factors such as light intensity and chemical and physical properties of the soil that result in good chlorophyll activity [18] could also explain the observed differences.

Table 6. Correlations bety	tween quantitative and o	chemical c	haracteristics
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	FLC	PEL	LIL	LIW	DIS	FWL	TEF	FWS	Ν	Р	K	Mg	Fe	Cu	Mn	Zn
FLC	1.00															
PEL	0.82**	1.00														
LIL	-0.38	-0.34	1.00													
LIW	0.76**	0.58	-0.76**	1.00												
DIS	-0.14	0.33	0.38	-0.24	1.00											
PWL	-0.44	0.01	0.33	-0.31	0.87**	1.00										
TEF	0.89**	0.73**	-0.50	0.87**	-0.01	-0.26	1.00									
FWS	-0.48	0.01	0.37	-0.4	0.90**	0.92**	-0.28	1.00								
Ν	-0.13	-0.09	0.72**	-0.41	0.38	0.38	-0.11	0.41	1.00							
Р	0.13	-0.30	0.34	0.01	-0.54	-0.43	-0.08	-0.53	0.26	1.00						
K	0.90**	0.68	-0.33	0.75	-0.18	-0.32	0.76**	-0.52	-0.17	0.32	1.00					
Mg	0.32	0.35	-0.48	0.34	0.10	-0.11	0.50	-0.08	-0.49	-0.68	0.16	1.00				
Fe	0.30	0.05	-0.59	0.36	-0.73**	-0.61	0.24	-0.71**	-0.24	0.11	0.30	0.14	1.00			
Cu	-0.393	-0.513	0.54	-0.43	0.20	0.28	-0.15	0.29	0.62	0.02	-0.41	0.05	-0.19	1.00		
Mn	-0.546	-0.506	0.56	-0.52	0.43	0.49	-0.36	0.46	0.17	-0.14	-0.44	0.17	-0.63	0.70	1.00	
Zn	0.435	0.258	-0.48	0.56	-0.08	-0.20	0.74**	-0.16	-0.06	-0.35	0.24	0.71	0.36	0.34	-0.04	1.00
ß-Cart	0.739**	0.683	-0.64	0.81	-0.08	-0.38	0.81**	-0.24	-0.40	-0.19	0.50	0.43	0.13	-0.49	-0.53	0.52

*: significant correlation to the threshold of 5%, **: significant correlation to the threshold of 1%, PEL: Petiole length; LIL: Limb length; LIW : Limb width; FLC: 50 % Flowering cyle; DIS: Diameter of the stem; FWL: Fresh weight of the leaves; FWS: Fresh weight of the stems; N: Nitrogen; P: Phosphorus; K: Potassium; Mg: Magnesium; Fe: Iron; Cu: Copper; Mn: Manganese; Zn: Zinc; ß-Car: ß-carotene; TEF: water content;

Although there were no significant differences in iron and ß-carotene content between the morphotypes, the SBL1 morphotype gave the highest levels of these elements. Similarly, the low dry matter content and the highest water content presented by this morphotype support the information given by the producers reported by [5]. Indeed, according to producers, the SBL1 morphotype with green stems and shiny lightgreen leaves called "shinyCorchorus" or "White Corchorus", referring to an improved variety, is generally consumed fresh because of its low dry matter content and high water content. The morphotype with red stems and dark green leaves called "bulvankmoaga" in the local Moore language, referring to a local species with a high dry matter content, is generally consumed dry.

The correlations between morphological traits and nutritional elements could guide breeders in improving the composition of nutritional elements in C. olitorius leaves. Indeed, the strong positive correlation between ß-carotene and potassium content shows that selection and improvement for ß-carotene content may indirectly lead to increased potassium content. In addition, the negative correlation between the number of days 50 % flowering and fresh leaf weight shows that late-cycle morphotypes have low leaf biomass. Similar results were reported by [5]), who showed that a shorter cycle could contribute to the improvement of the yield of this leafy vegetable. However, this could lead to a decrease in nutritional quality, as the cycle is positively and strongly correlated with the main mineral elements (K, Mg, Fe, Zn) and ß-Carotene content. Indeed, the strong correlation between ß-carotene content and the number of days 50 % flowering suggests that the long-cycle morphotypes were able to express their photosynthetic activity at best, thus allowing an accumulation of carotenoid pigments. In addition, the strong positive influence of leaf blade width on ß-carotene and potassium contents would reflect a more intense photosynthetic activity of the genotypes with wide leaf blades which would have resulted in a strong synthesis and accumulation of these two elements. [17], also observed a positive correlation between leaf area and potassium content.

The values of genetic parameters especially the low difference between phenotypic and genotypic coefficients of variation at the level of all traits and the high values of high broad-sense heritability of most of the traits except for stem diameter, stem fresh weight, copper, magnesium, nitrogen and iron content would indicate a weak influence of the environment on expression of most of the studied the parameters. Thus, the phenotype could allow a good prediction of the genotype. Similar results were reported on Corchorus olitorius bv[14] and on Cleome gynandra by [13]. Furthermore, the high genetic advance (GAx) for leaf-related traits and number of days 50 % flowering would indicate a weak influence of environmental effect on the expression of these traits. Indeed, according to [19], when genetic progress is high, heritability is mainly due to the effect of additive genes. Moreover, according to [20], when a trait is under the effect of additive genes, it allows a good prediction of the genotype. For this, a direct selection method for the C. olitorius could be based on these traits. But the plant height, number of primary branches, fresh leaf weight, potassium content, phosphorus content and water content, recorded very high heritability values coupled with relatively low genetic gains. This would show that high heritability is not necessarily associated with high genetic gain. Indeed, according to[21], heritability in the broad sense includes, in addition to heritable additive genetic variance, non-additive variance due to epistasis that not dominance and are transmissible. Thus, the low coefficient of genotypic variation (GCV) associated with the low genetic gain (GAx), observed for all biochemical traits would reflect a strong pressure of environmental effect on these traits. Previous studies [22], have also reported the influence of cultural practices, notably the addition of organic and chemical fertilizers and the use of pesticides and herbicides on the variation of biochemical composition.

4. CONCLUSION

This study showed a very high content of nutritional elements (iron, zinc, potassium, phosphorus and ß-carotene) in the leaves of C. olitorius. This content is very little dependent on the morphotype and the botanical variety. The SBL1 morphotype with green stems and greenlight leaves showed the highest ß-carotene and iron content. In addition, positive correlations between leaf blade width and the traits ßcarotene content and potassium content were observed. As morphological and biochemical are generally influenced parameters by environmental factors, it would be wise to confirm these results by conducting multi-location and multi-year trials. It would also be important to study the effect of organic and mineral fertilizer doses on the parameters studied.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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