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MORPHOLOGICAL CHARACTERIZATION OF *OROBANCHE CRENATA* IN CARROTS AND LEGUMES (FABA BEAN AND CHICKPEA): INDICATIONS OF POTENTIAL GENETIC DIFFERENTIATION TOWARDS HOST PLANTS

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ABSTRACT

Carrot is an important root vegetable crop in Morocco. Recently, it has been reported to have been attacked by the parasitic plant Orobanche crenata*, which is known for infesting legumes. The aim of this study is to provide a morphological comparison between carrot-hosted, and faba bean- and chickpea-hosted* O. crenata *plants according to quantitative and qualitative parameters. High genetic variability was observed in the qualitative traits of parasitic plants at intra- and inter-host levels. In fact, differences in the color of stem, leaves, petals, calyx, sepals, central bract of flowers, stigma lobes, and anthers were observed. In contrast, the variability of quantitative traits was low. For instance, variation between parasitic plants by type of host crop was only significant in collar circumference and sucker circumference. The results of this study could be tentatively explained by host-parasite specific interaction and adaptation.*

Key words: Carrot, chickpea, faba bean, genetic variability, morphological comparison, *Orobanche crenata*

CARATTERIZZAZIONE MORFOLOGICA DI *OROBANCHE CRENATA* IN CAROTE E LEGUMI (FAVE E CECI): INDICAZIONI DI POTENZIALE DIFFERENZIAZIONE GENETICA A SECONDA DELLE PIANTE OSPITANTI

SINTESI

La carota è un importante raccolto di ortaggi a radice in Marocco. Recentemente è stato segnalato che è stata attaccata dalla pianta parassita Orobanche crenata*, nota per infestare i legumi. Lo scopo di questo studio è quello di fornire un confronto morfologico tra piante di* O. crenata *ospitate da carote e da piante di fave e ceci, secondo parametri quantitativi e qualitativi. È stata osservata un'elevata variabilità genetica nei tratti qualitativi delle piante parassite a livello di intra- e inter-ospite. Sono state infatti osservate differenze nel colore del fusto, delle foglie, dei petali, del calice, dei sepali, della brattea centrale dei fiori, dei lobi dello stigma e delle antere. Al contrario, la variabilità dei tratti quantitativi è risultata bassa. Ad esempio, la variazione tra le piante parassite per tipo di coltura ospite era significativa solo nella circonferenza del colletto e nella circonferenza della ventosa. I risultati di questo studio potrebbero essere provvisoriamente spiegati dall'interazione e dall'adattamento specifici per il rapporto ospite-parassita.*

Parole chiave: Carota, ceci, fave, variabilità genetica, confronto morfologico, *Orobanche crenata*

INTRODUCTION

The carrot (*Daucus carota* L.) is a biennial dicotyledonous plant belonging to the Apiacea family. This family includes approximately 445 genera and 3700 species (Downie & Katz-Downie, 1996). The carrot is cultivated in all temperate regions of the world and even in subtropical zones during the fresh season (Chaux & Foury, 1994). It is considered among the ten most important vegetable crops in the world, in terms of production area (about 1.2 million hectares) and market value (Simon *et al.*, 2008). The carrot is cultivated for its roots, used as human food for its richness in carotenoids, -carotene in particular, which protects from vision disturbances and maintains the good condition of the skin and other parts of the organism. It contains high levels of carbohydrates and other vitamins (B, C, K). Carrot root is widely used in children nutrition thanks to its fiber content adapted to the consumption methods (salads, fresh, cooked, juices, etc.) (Villeneuve, 1999).

The carrot plant is part of the host range of *Orobanche crenata*. This parasitic plant threatens carrot farming especially in the Mediterranean region including North Africa, the Near East and western Asia, with quite recent introductions into Sudan and Ethiopia (Babiker *et al.*, 2007). It infests the most important legume crops of the Mediterranean, particularly faba bean, lentil, chickpea, vetch, and field pea, but also carrot (Schaffer *et al.*, 1991). It reduces the yield and destroys the quality of carrot root (Schaffer *et al.*, 1991). Recently in Morocco, there have been reports of carrot fields (9000 ha) infested by *O. crenata*. The infested areas are Chaouia (*north*-central *Morocco*) and south of Doukkala (north-western Morocco), accounting for more than 60% of the acreage of the carrot crop. Meanwhile, no infestation has been reported in the remaining cultivation areas: Gharb (western Morocco), Tadla (central Morocco), Sous (central-south Morocco), and Sais (Chedadi *et al.*, 2019). *O. crenata* causes significant yield decreases ranging from 15 to 21%. The economic loss has been estimated to run between 2600 and 3400 USD/ha (Chedadi *et al.*, 2020).

O. crenata belongs to the genus of *Orobanche*. This genus contains more than 150 species (Musselman, 1980), but only five of them are considered dangerous for agricultural production, namely, *O. crenata, O. cumana, Phelipanche aegyptiaca, Phelipanche ramosa*, and *O. minor* (Perez-de-Luque *et al.*, 2004). The differentiation between these species is based on morphological criteria (stem, leaves or bracts, flower, hairiness, stigma, anthers, corolla, stigma lobes, etc.) as described in plant identification and taxonomic keys (Parker, 2013; Foley, 2000). There is a wide genetic variability within broomrape populations of the same species in the color or size of the corolla, degree of pubescence, and some other morphological characters (Musselman, 1994). This variability can be influenced by the host range and preference host (Joel, 2000). *O. crenata* populations exhibit high variability in several characters: color of stem, flowers, and stigma, density of flowers on the stem, and size of plant (length and diameter) (Cuebro, 1979). The variability in *O. crenata* is not only morphological, it is also associated with parasitism; in fact, host-parasite interaction effects have been observed that have quantitative impact on the variability (Abdallah & Darwich, 1994). For instance*, O. crenata* plants can reach greater vegetative vigor and height in faba beans and produce 10 times more seeds than in lentils (Kroschel, 2001). The *O. crenata* height can range from 30 to 100 cm. The plant has a hairy stem with leaves reduced to bracts. The bracts are simple and alternate, their upper and lower surfaces hairy. The inflorescence is a spike carrying flowers of 1.5 to 3 cm in length. Petals are fused and hairy. The color of the corolla varies from yellowish white to purple (Servais & Seba, 2020).

The morphological characteristics are important parameters allowing easy identification of genetic diversity in inter- and intra-population studies. Most qualitative traits have been reported as inherited following monogenic models in different plants, thus providing useful information for the description and identification of biological material and the classification of the species, as well as for the comparison of individuals within the same species (Andersson *et al.*, 2006).

To our knowledge, the available literature contains no published reports of comparisons between *O. crenata* plants attacking carrots and those attacking legumes. Thus, our study aims at providing a morphological comparison between plants of *O. crenata* hosted by two legumes, faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*), and those hosted by the carrot, using quantitative and qualitative descriptive parameters to investigate the differences and similarities between them and to explore the hypothesis of possible genetic differentiation according to the host plant.

MATERIAL AND METHODS

For comparing legume-hosted and carrot-hosted *O. crenata* plants, a total of 90 plants of *O. crenata* were collected from carrot, faba bean and chickpea fields in the Chaouia area, located in the Casablanca-Settat region (33° 32′ 00″ North, 7° 35′ 00″ West of Morocco). For each crop, 10 plants were randomly collected from three different fields.

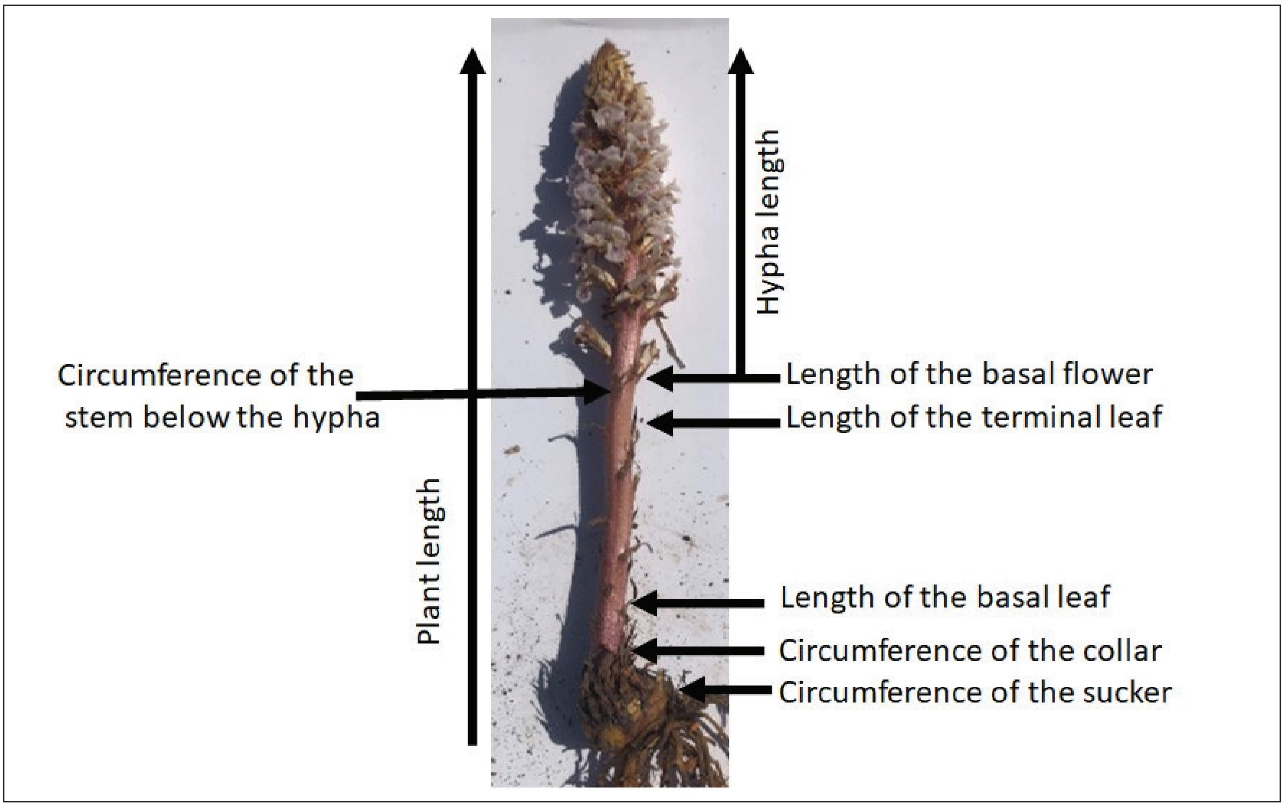

Fig. 1: Illustration of quantitative parameters measured in *O. crenata* ***plants for the purpose of morphological description.***
Sl. 1: Izmerjeni kvantitativni parametri pri rastlinah *O. crenata* ***za morfološki opis.***

The morphological characterization of the collected *O. crenata* plants was carried out based on both quantitative and qualitative traits according to the descriptor Tilo botanica (Servais & Seba, 2020). The measured quantitative characters (Fig. 1) included: plant height (PH), sucker circumference (SC), collar circumference (CC), stem circumference below the hypha (CTH), length of basal leaf (LFB) and terminal leaf (LFT), basal flower length (LFBA), hypha length (LH), number of anthers (NE), and number of stigma lobes (NLS).

The studied qualitative characters included the color of stem, leaves, petals, calyx, sepals, top of the central bract, base of the central bract, sides of the central bract, anthers, stigma of lobes, and the presence or absence of hair at anthers, petals, sepals and ovary filaments.

The descriptive data analysis was performed using Excel 2016 for each trait. Averages, standard deviations, coefficients of variation, and frequency of distribution were calculated. To estimate the extent of morphological diversity and differentiation among and between *O. crenata* populations, morphological diversity (MD) based on Simpson index (1) and phenotypic differentiation (PD) (2) index between each two consecutive *O. crenata* populations were calculated for the qualitative traits using the following formulas:

$$(1)\ \mathbf{MD} = \mathbf{1} - \sum_{\mathbf{1}}^{s} (pi)^2$$

pi is the proportion of the total number of individuals in the population with the i^{th} morphological character state, and s is the total number of morphological character states (Casas *et al.*, 2006; Blancas *et al.*, 2009).

$$(2)\ \mathbf{PD_{1-2}} = \mathbf{ln}\frac{\mathbf{xiyj}}{\sqrt{\left(\sum_{1}^{s1}(\mathrm{xi})^2 \sum_{1}^{s2}(\mathrm{yj})^2\right)}}$$

xi and yj are frequencies of individuals with the i^{th} and j^{th} morphological character state in populations 1 and 2, s_1 and s_2 are the total number of morphological character states for a given qualitative trait in the two respective populations, ln is the Napierian logarithm (Blancas *et al.*, 2009).

For each *O. crenata* population and each trait, the average morphological diversity index was calculated. Average phenotypic differentiation between pairwise populations was estimated for each trait.

Tab. 1: Average, minimum, maximum, and coefficient of variation of quantitative parameters measured in* O. crenata *host plants: carrot, chickpea, and faba bean.
Tab. 1: Povprečje, minimum, maksimum in koeficient variacije kvalitativnih parametrov, izmerjenih pri gostiteljih pojalnikov* O. crenata*: korenju, čičeriki in bobu.

Host crops		PH (cm)	SC (cm)	CC (cm)	CTH (cm)	LFB (cm)	LFT (cm)	LH (cm)	LFBA (cm)
Carrot	A.	47.7a±9.86	9a±2.17	3.9a±0.70	3.2a±0.67	2a±0.36	1.7a±0.37	20.7a±7.08	2.3a±0.28
	Min	28	5.1	2.8	1.9	1.5	1	10	1.9
	Max	66.4	16	5.5	5.1	3.2	2.5	37	3
	CV	21%	24%	18%	21%	17%	22%	34%	12%
Faba bean	A.	44.9a±12.59	7.1b±1.95	3.3b±0.62	3a±0.59	2a±0.30	1.7a±0.27	23.7a±8.73	2.5a±0.53
	Min	27.1	4.6	2.5	2.3	1.5	1.1	8.6	1.1
	Max	84.5	14.6	4.9	4.9	3	2.2	43.9	3.4
	CV	28%	27%	19%	19%	15%	16%	37%	22%
Chickpea	A.	44.3a±9.30	6.9b±1.63	3.4b±0.53	3.1a±0.43	1.9a±0.32	1.7a±0.68	25a±7.33	2.6a±0.47
	Min	21.2	3.3	2.4	2.4	1.2	1.1	9.9	1.1
	Max	62.5	10.2	4.8	3.8	2.6	2.2	37.9	3.2
	CV	21%	24%	16%	13%	17%	16%	29%	18%

Considering all the characters, a discriminant factor analysis, using SPSS 19.0 for Windows, was performed to test the hypothesis of genetic differentiation in the studied *O. crenata* populations according to their membership to the predefined groups, which corresponded to classes of host plant (carrot, faba bean, and chickpea). All recorded characteristics of *O. crenata* plants were considered as descriptive variables, and the crop class as the explained variable. The corresponding eigenvalues of discriminant functions, significant levels, and correlations to descriptive variables were analyzed and a graphic representation of the collections was produced to display the obtained results. For further confirmation and comparison, a hierarchical classification of different *O. crenata* accessions, based on Nei & Li's (1979) genetic distance from a binary matrix of presence/absence of descriptive qualitative trait classes, was carried out using TREECON for Windows (Vandepeer & Dewachter, 1994). Bootstrap analysis with 100 iterations was used to test the confidence and faithfulness of the obtained groupings.

RESULTS

Variation in quantitative traits

Averages, minimum, maximum, and coefficients of variation were reported for quantitative traits (Tab. 1). Significant variation was observed in the circumference of the sucker between legume- and carrot-hosted plants. In the the two legumes, faba bean and chickpea, it was almost identical, with averages of 7.1 and 6.9 cm, respectively, while in carrot-hosted *O. crenata* the average was higher by 2 cm, namely 9 cm (Fig. 2). Significant variation was also observed in the circumference of the collar. Although the difference was not statistically significant (F=0.09), the hypha length (LH) value in *O. crenata* plants collected in carrot fields was lower than in plants from faba bean and chickpea fields (20.7, 23.7, and 25 cm, respectively). Likewise the plant height: although the differences by crop type regarding this parameter were not statistically significant, *O. crenata* collected in carrot fields were taller, averaging about 47.7 cm, than those collected in faba bean and chickpea fields, averaging about 44.9 and 44.3 cm, respectively (Tabs. 1 & 2).

With regard to other quantitative parameters (number of anthers and number of stigma lobes), the plants of *O. crenata* exhibited the same values, 4 and 2, respectively, regardless of the host plant.

Variation in qualitative traits

Color of leaves

During our survey, we observed three colors of leaves among the studied *O. crenata* population col-

Tab. 2: Variance analysis of* O. crenata *quantitative traits according to the three host crops. Means significantly different at P ≤ 0.05. Plant height (PH), sucker circumference (SC), collar circumference (CC), stem circumference below the hypha (CTH), length of the basal leaf (LFB) and terminal leaf (LFT), hypha' length (LH), basal flower length (LFBA).
Tab. 2: Analiza variance kvantitativnih znakov pojalnikov (*O. crenata*) glede na tri gostiteljske kulture. Srednje vrednosti so statistično značilne na nivoju P ≤ 0.05. Višina rastline (PH), obseg sesalnih korenin (SC), obseg ovratnika (CC), obseg stebla pod hifo (CTH), dolžina bazalnega (LBF) in terminalnega lista (LFT), dolžina hife (LH) in dolžina bazalnega cveta (LFBA).

Source of variation	Traits	Sum of squares	Degree of freedom	Mean squares	F	Signification *
Treatment (host crop)	**PH**	193.8	2	96.9	0.82	0.44
Error		10173.4	87	116.9		
Total		10367.2	89			
Treatment (host crop)	**SC**	85.2	2	42.6	11.2	0.0001
Error		330.7	87	3.8		
Total		416	89			
Treatment (host crop)	**CC**	5.87	2	2.6	6.8	0.002
Error		34.3	87	0.3		
Total		39.7	89			
Treatment (host crop)	**CTH**	0.69	2	0.34	1.04	0.35
Error		29	87	0.33		
Total		29.7	89			
Treatment (host crop)	**LFB**	0.41	2	0.2	1.91	0.15
Error		9.48	87	0.1		
Total		9.9	89			
Treatment (host crop)	**LFT**	0.007	2	0.003	0.036	0.965
Error		8.3	87	0.096		
Total		8.3	89			
Treatment (host crop)	**LH**	294.8	2	147.4	2.3	0.09
Error		5353.9	87	61.5		
Total		5648.7	89			
Treatment (host crop)	**LFBA**	1.15	2	0.5	2.8	0.06
Error		17.9	87	0.2		
Total		19	89			

lected from the three crops (carrot, faba bean and chickpea): dark brown, brown, and light brown. All three colors were found in *O. crenata* plants in carrots and faba beans, while those in chickpeas only displayed the brown and light brown colors. *O. crenata* plants with brown leaves were the most frequent, with a frequency of 83%, while dark brown and light brown leaves were present at frequencies of 6% and 11%, respectively (Tab. 3).

Stem color

The color of the stem varied in all three crops (Tab. 4, Fig. 3), from dark purple, purple and light purple to pale yellow. The respective frequencies of the colors were 28, 42, 18 and 12%. *O. crenata* plants with purple and dark purple stems were the most frequent among the population studied, with a frequency of about 70%.

Fig. 2: Different circumferences of the sucker observed among the studied** O. crenata **populations in carrot crops.
Sl. 2: Različni obsegi sesalnih korenin pri raziskanih pojalnikih** O. crenata **v kulturah korenja.

Tab. 3: Frequency distribution of leaf color among the studied** O. crenata **populations.
***Tab. 3: Frekvenčna porazdelitev barve listov raziskanih pojalnikov vrste** O. crenata.*

	Carrot	Faba bean	Chickpea	Total
Dark brown	13%	3%	0%	6%
Light brown	13%	13%	7%	11%
Brown	73%	83%	93%	83%

Tab. 4: Frequency distribution of stem color among the studied** O. crenata **populations.
Tab. 4: Frekvenčna porazdelitev barve stebla raziskanih pojalnikov vrste** O. crenata**.

	Carrot	Faba bean	Chickpea	Total
Dark purple	30%	23%	30%	28%
Purple	47%	33%	46%	42%
Light purple	10%	27%	17%	18%
Pale-yellow	13%	17%	7%	12%

Fig. 3: Different stem colors observed among the studied* O. crenata *populations in carrot crops.
Sl. 3: Različna obarvanost stebla pri raziskanih pojalnikih* O. crenata *v kulturah korenja.

Tab. 5: Frequency distribution of corolla color among the studied *O. crenata* ***populations.***
Tab. 5: Frekvenčna porazdelitev barve cvetnega odevala raziskanih pojalnikov vrste *O. crenata****.***

	Carrot	Faba bean	Chickpea	Total
White striped purple	47%	33%	46%	42%
White striped dark purple	30%	23%	30%	28%
White striped light purple	10%	27%	17%	18%
Off-white	13%	17%	7%	12%

Tab. 6: Frequency distribution of sepal color among the studied *O. crenata* ***populations.***
Tab. 6: Frekvenčna porazdelitev barve čašnih listov raziskanih pojalnikov vrste *O. crenata****.***

	Carrot	Faba bean	Chickpea	Total
Dark purple	30%	23%	30%	28%
Purple	44%	33%	47%	41%
Light purple	13%	27%	17%	19%
Pale-yellow	13%	17%	6%	12%

Fig. 4: Color of flower components (calyxes, sepals, central bract, petals, and stigma lobes) observed among the studied *O. crenata* ***populations.***
Sl. 4: Barva cvetnih elementov (čaše, venčni in cvetni listi, brakteje in brazde) pri raziskanih pojalnikih *O. crenata* ***v kulturah korenja.***

Corolla color

Four different colors of flower petals were observed among the plants of *O. crenata* (Tab. 5; Figs. 4 & 5): white petals with purple streaks, purple with dark or light purple streaks, and off-white petals. The frequency distribution among the three classes of crops was 47%, 30%, 10%, and 13%; 33%, 23%, 27% and 17%; and 46%, 30%, 17%, and 7% for populations from carrot, faba bean and chickpea fields, respectively.

During our investigation, we noticed significant association between stem color and petal color. In fact, all dark purple stemmed plants had white petals streaked with dark purple, purple stemmed ones had white petals streaked with purple, light purple stemmed plants had white petals streaked with light purple, and in pale yellow stemmed plants the petals were off-white.

Color of sepals

The analysis of the observed proportions showed that the sepals displayed the same colors but with different frequencies in different crops. Their total frequencies were 28% for dark purple, 41% for purple, 19% for light purple, and 12% for pale yellow (Tab. 6).

Central bract of flower

The central bract of the flower lacked a uniform color, instead, it displayed several colors of differ-

Fig. 5: Variability in *O. crenata* ***flowers and stems observed in a single carrot field in Morocco (Chaouia region).***
Sl. 5: Variabilnost cvetov in stebel *O. crenata* ***iz nasada korenja v Maroku (predel Chaouia).***

ent intensity between its top, base and sides. With regard to the top of the central bract (Tab. 7), up to five different colors were recorded: dark brown, brown, pale yellow, dark purple, and purple. The commonest among the three studied populations were *O. crenata* plants with a brown (54%) and purple (33%) central bract top. A purple central bract top was the most frequent in *O. crenata* populations from chickpea fields. The dark brown color was only observed in *O. crenata* plants from carrot fields; the same population lacked individuals with purple and dark purple central bract top.

Tab. 7: Frequency distribution of color of the top of the central bract among the studied* O. crenata *populations.
***Tab. 7: Frekvenčna porazdelitev vrha osrednje brakteje pojalnikov vrste* O. crenata.**

	Carrot	Faba bean	Chickpea	Total
Dark brown	3%	0%	0%	1%
Brown	87%	60%	17%	54%
Pale-yellow	10%	13%	3%	9%
Dark purple	0%	4%	3%	3%
Purple	0%	23%	77%	33%

Tab. 8: Frequency distribution of color of the base of the central bract among the studied* O. crenata *populations.
***Tab. 8: Frekvenčna porazdelitev barve baze osrednje brakteje raziskanih pojalnikov vrste* O. crenata.**

	Carrot	Faba bean	Chickpea	Total
Purple	13%	13%	20%	16%
Pale-yellow	87%	77%	77%	80%
Brown	0%	3%	0%	1%
White	0%	7%	3%	3%

With regard to the base of the central bract (Tab. 8), four colors were observed: purple, pale yellow, brown, and white. The commonest was pale yellow (80%), followed by purple (16%), while the brown and white colors were rare. The brown color was only found in *O. crenata* populations from faba bean fields; white and brown were absent in populations from carrot fields.

Four different colors were identified with regard to the sides of the central bract (Tab. 9): dark purple, light purple, pale yellow, and brown. *O. crenata* plants with pale yellow and brown lateral parts of the central bract accounted for more than 90% of the studied populations, the dark purple and light purple ones for less than 10%. The latter two colors were absent in *O. crenata* plants from chickpea fields, the light purple in those from carrot fields. The carrot-hosted *O. crenata* populations exhibited a higher share of pale yellow (70%).

Stigma lobe color

The stigma lobes of the studied *O. crenata* populations exhibited multiple colors (Tab. 10; Fig. 4); we observed up to six of them: dark purple, purple, brown, light brown, yellow, and black. The total frequencies of these colors were, respectively, 12%, 27%, 34%, 1%, 25%, and 1%. Light brown and black stigma lobes were not present in *O. crenata* populations from chickpea and carrot fields.

Tab. 9: Frequency distribution of the color of the sides of the central bract among the studied* O. crenata *populations.
***Tab. 9: Frekvenčna porazdelitev barve strani centralne brakteje raziskanih pojalnikov vrste* O. crenata.**

	Carrot	Faba bean	Chickpea	Total
Dark purple	17%	3%	0%	7%
Pale-yellow	70%	37%	37%	48%
Brown	13%	57%	63%	44%
Light purple	0%	3%	0%	1%

Anther color

The colors observed in anthers were brown, black, dark gray, and purple (Tab. 11). *O. crenata* plants with black anthers represented more than 50% of all studied populations. The purple color was rare and represented only 6% of all individuals. It was not found in populations from carrot fields.

Plant pubescence

All *O. crenata* specimens collected from the three different crops displayed pubescence in different parts of the plant: leaves, petals, sepals, ovary filaments, and stamens.

Morphological diversity and phenotypic differentiation of qualitative traits

The morphological diversity index ranged from 0.2311 in relation to the color of the base of the

Tab. 10: Frequency distribution of stigma lobe color among the studied* O. crenata *populations.
***Tab. 10: Frekvenčna porazdelitev barve brazd raziskanih pojalnikov vrste* O. crenata.**

	Carrot	Faba bean	Chickpea	Total
Dark purple	26%	10%	0%	12%
Purple	40%	13%	27%	27%
Brown	17%	37%	50%	34%
Light brown	0%	3%	0%	1%
Yellow	17%	34%	23%	25%
Black	0%	3%	0%	1%

central bract to 0.7133 in the stigma lobe color in the carrot-hosted *O. crenata* population, with an average of 0.5382. It ranged from 0.2867 in relation to leaf color to 0.7356 for the four following traits: stem color, corolla color, calix color, and sepal color in the faba bean-hosted *O. crenata* population, with an average of 0.6100, the highest among the three populations. In the chickpea-hosted population, the average was 0.5127, with values ranging from 0.1244 to 0.6600 (Tab. 12).

The highest phenotypic differentiation indices were observed in carrot-hosted *O. crenata* populations, with average values of 0.2447 between carrot versus chickpea populations, followed by 0.1317 between carrot versus faba bean populations. While in legume pairwise populations (faba bean-chickpea), the average phenotypic differentiation index observed was only 0.0978. Higher differentiation indices were observed in relation to the following traits in the carrot-legume pairwise (Tab. 12): color of the lateral parts of the flower's central bract, stigma lobe color, anther color, and color of the top of the central bract.

Discriminant factor analysis and hierarchical classification

These two analyses were carried out to test the hypothesis of differentiation of individuals in *O. crenata* populations from separate groups. The two discriminating functions of the analysis were significant. The first discriminating function was highly significant (Wilks Lambda: 0.218; Chi-square: 117.966; P: 0.000) with an eigenvalue of 2.60 explaining 90.5% of the total variability and corresponding to a correlation of 0.85. A mild, yet significant genetic differentiation was observed in the studied morphological characters for *O. crenata* from carrot fields compared to those from legume-planted (faba bean, chickpea) fields (Fig. 6).

The second discriminating function was correlated with the following explanatory variables: color of anther, color of the top central bract, length of basal flower, circumference of collar, circumference of sucker, and length of inflorescence. The dif-

Tab. 11: Frequency distribution of anther color among the studied* O. crenata *populations.
Tab. 11: Frekvenčna porazdelitev barve prašnikov raziskanih pojalnikov vrste* O. crenata*.

	Carrot	Faba bean	Chickpea	Total
Brown	13%	33%	7%	18%
Black	40%	47%	73%	53%
Dark gray	47%	13%	10%	23%
Purple	0%	7%	10%	6%

Tab. 12: Morphological diversity and phenotypic differentiation of qualitative traits among the studied* O. crenata *populations.
Tab. 12: Morfološka raznolikost in fenotipska diferenciacija kvalitativnih znakov med populacijami pojalnika vrste* O. crenata*.

Crops	Leaf color	Stem color	Corolla color	Calyxes' color	Sepals' color	Top part of the central bract of the flowers color	Down part of central bract of the flowers color	Side parts of the flowers' central bract color	Stigma' lobes color	Stamens' anther color	Average
Morphological diversity index											
Carrot	0.4267	0.6578	0.6644	0.6867	0.6867	0.2378	0.2311	0.4733	0.7133	0.6044	0.5382±0.18
Faba bean	0.2867	0.7356	0.7356	0.7356	0.7356	0.5667	0.3889	0.5422	0.7244	0.6489	0.6100±0.16
Chickpea	0.1244	0.6600	0.6600	0.6600	0.6600	0.4644	0.3711	0.4644	0.6244	0.4378	0.5127±0.17
Phenotypic differentiation index											
Carrot-faba bean	0.0097	0.0639	0.0820	0.0558	0.0558	0.0747	0.0048	0.3741	0.3570	0.2394	0.1317±0.10
Carrot-chickpea	0.0217	0.0164	0.0132	0.0094	0.0094	1.2668	0.0061	0.4427	0.3601	0.3013	0.2447±0.39
Faba bean-chickpea	0.0046	0.0489	0.0645	0.0645	0.0645	0.4961	0.0053	0.0037	0.0810	0.1447	0.0978±0.14

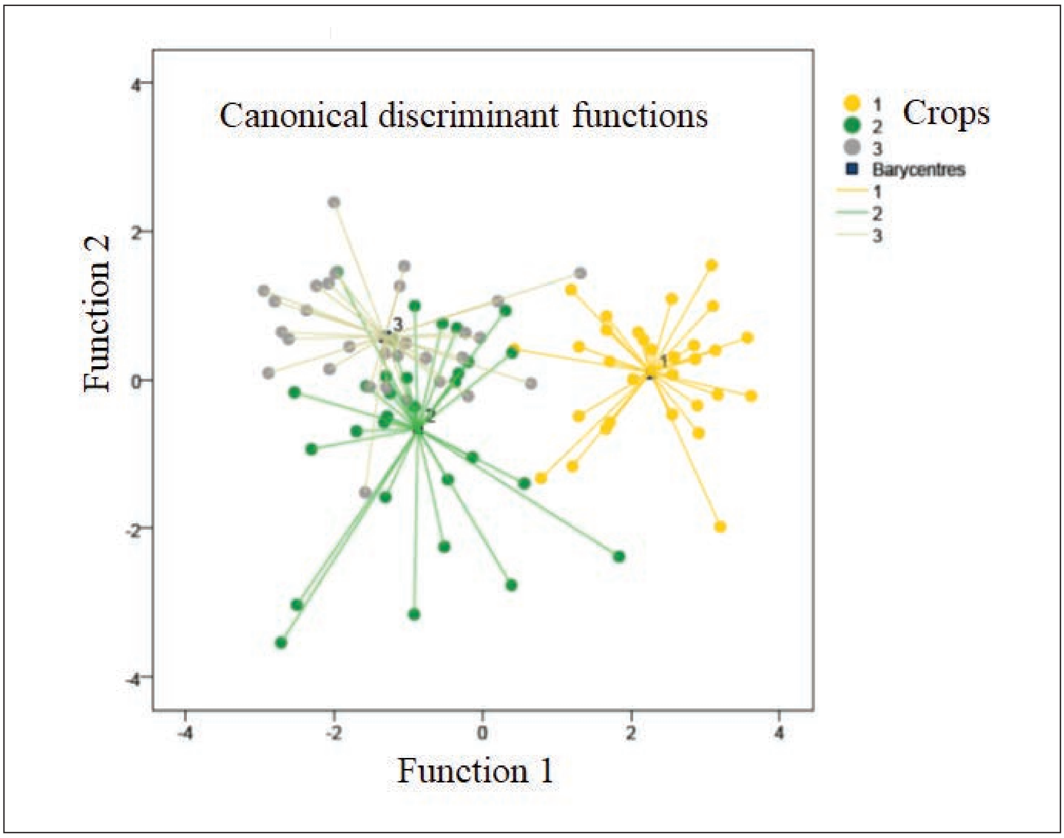

Fig. 6: Genetic differentiation among** O. crenata **populations according to the host plant: carrot (1), faba bean (2), chickpea (3).
Sl. 6: Genetska diferenciacija znotraj populacij pojalnikov vrste** O. crenata **glede na vrsto gostitelja: korenje (1), bob (2), čičerika (3).

ferentiation observed is largely explained by these variables.

With regard to the hierarchical analysis, distances in qualitative traits in all individuals from the three populations corresponding to the three host crops were used to build a classification tree showing similarities between these individuals. Although no separate clustering by host crop was obtained, six different groups were observed (Fig. 7). Interestingly, two of these groups were largely composed of *O. crenata* accessions hosted by carrot (G2 and G4).

G2 had a moderately high bootstrap value of 68 and contained exclusively 11 accessions of carrot-hosted *O. crenata*, and G4 was composed of two subgroups also containing exclusively 9 accessions of *O. crenata* collected from carrot fields. The two subgroups had high bootstrap values of 84 and 95. Another small subgroup of four *O. crenata* accessions from carrot fields were clustered together in group G6 with a bootstrap value of 85. The total of differentiated carrot-hosted *O. crenata* accessions accounted for 79% of the population.

DISCUSSION

Statistical analyses of different morphological parameters (qualitative and quantitative) showed slight genetic differences between carrot-hosted populations of *O. crenata* and those hosted by faba bean and chickpea. This was highlighted by moderate but significant genetic differentiation associated with host plants, displayed through functional discriminant analysis and hierarchical clustering that were based on genetic distances from qualitative traits. A morphological comparison of carrot-hosted *O. crenata* populations with those hosted by faba bean and chickpea showed a mild difference based on several morphological characters. We found that populations of *O. crenata* collected from carrot fields presented greater average plant height, circumference of the sucker, circumference of the collar, circumference of the stem below the hypha, and length of the basal leaf compared to populations collected from faba bean and chickpea fields. Moreover, further morphological differences between these populations (carrot-hosted and legume-hosted)

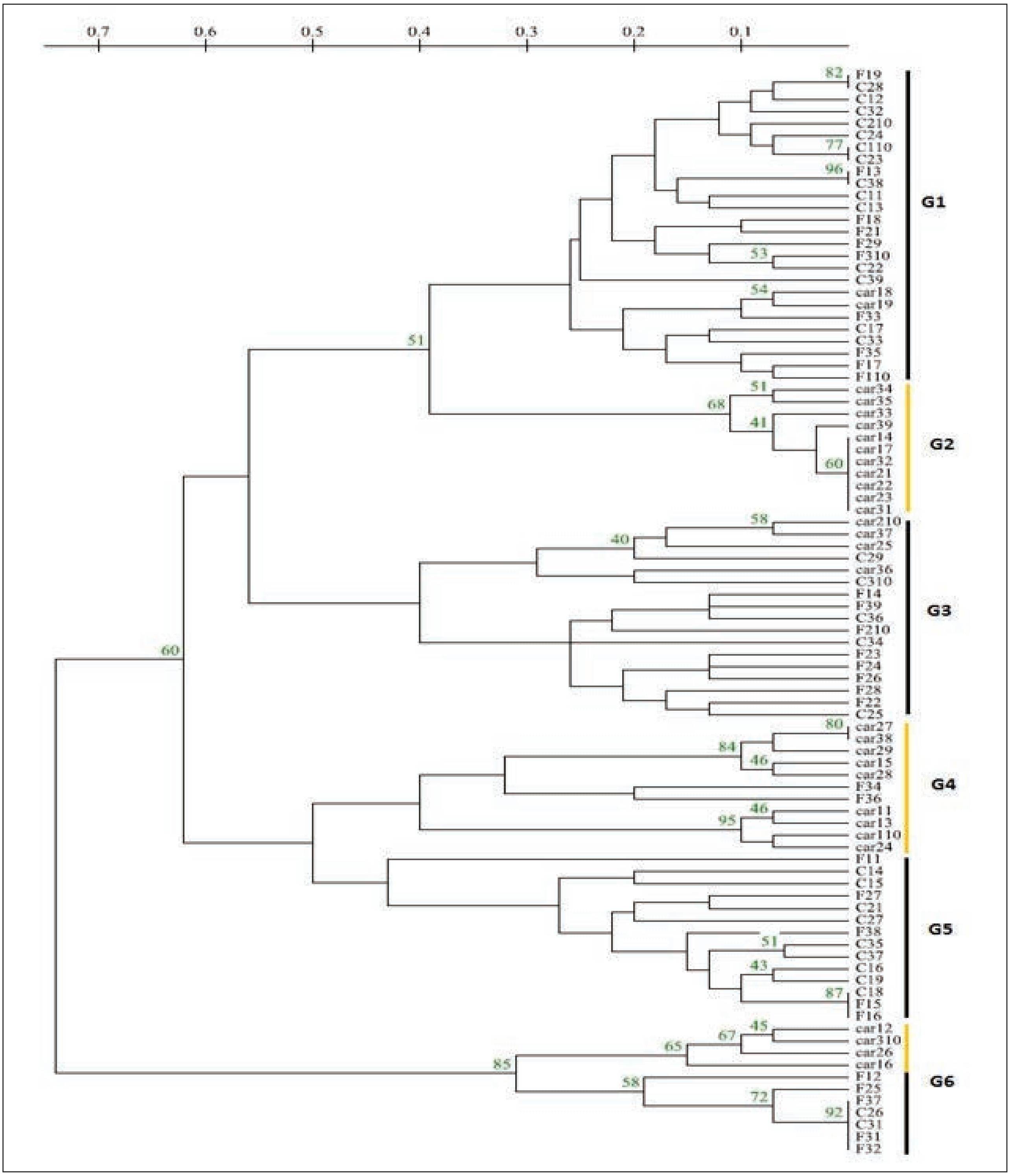

Fig. 7: Association between** O. crenata **accessions as revealed by the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on Nei and Li's (1979) genetic distance calculated from qualitative descriptive traits. Bootstrap values are given at the nodes. Continuous lines right of the figure indicate groups; orange color corresponds to carrot-hosted** O. crenata **accessions clustered together. F: faba bean, C: chickpea, and car: carrot.
Sl. 7: Povezava med zajedanjem vrste** O. crenata **na podlagi metode neuravnoteženih parov skupin z klaster-sko analizo aritmetične sredine (UPGMA) na podlagi genetske distance, izračunane na podlagi kvalitativnih opisnih znakov po Nei & Li's (1979).

were observed. With regard to the color of the top of the central bract, dark brown was only present in populations from carrot fields, while purple and dark purple tops of the central bract, white bases of the central bract, and white and purple anthers were only observed in faba bean- and chickpea-hosted populations. This potential genetic differentiation was also indicated by the highest phenotypic differentiation indices obtained for carrot-faba bean and carrot-chickpea pairwise populations, especially in relation to the above cited traits, in which the difference was obvious.

Morphological characterization showed a genetic variability in *O. crenata* plants collected from three different crops (carrot, faba bean, and chickpea) of the same region, and even within the same crop. This could be explained by the presence of different ecotypes of the species *O. crenata*. Domina (2018) confirmed this result, observing a variation in shape and size of flowers, number of flowers, density of the inflorescence, shape of the calyx, and color of the corolla and stigma of *O. crenata* from fields cultivated with faba bean, chickpea, *Pisum sativum*, and *Lathyrus clymenum*. Also, Joel (2000) mentioned that variability can be influenced by the host range and preference host. Our results are in agreement with those of Román *et al*. (2001), which were based on molecular analysis. These authors found a polymorphism rate of 91% while using RAPD markers to study the genetic diversity of six populations of *O. crenata* collected from faba bean fields on different sites located in the south of Spain (Andalusia). Employing the same molecular techniques, Ennami *et al*. (2017) detected genetic diversity in six *O. crenata* populations collected from faba bean and lentil fields in three regions of Morocco (Taza, Meknes ,and Settat). They showed an intra-population variability of 81% and 82% for populations in faba beans and lentils, respectively.

CONCLUSIONS

The study demonstrated intra- and inter-population genetic variability of *O. crenata* hosted by three different crops, which was expressed through different morphological characters. *O. crenata* plants collected from carrot fields were genetically slightly different from those collected from among legumes. Further DNA analyses of these populations to confirm the obtained results are under way.

MORFOLOŠKA OPREDELITEV VRSTE *OROBANCHE CRENATA* PRI KORENJU IN STROČNICAH (BOB IN ČIČERIKA): POKAZATELJI MOŽNE GENETSKE DIFERENCIACIJE PROTI GOSTITELJSKIM RASTLINAM

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POVZETEK

Korenje je pomembna kultura v Maroku. Pred kratkim so ugotovili, da ga napada zajedavska rastlina Orobanche crenata*, ki je sicer znana, da napada stročnice. Namen te raziskave je narediti morfološko primerjavo med rastlinami korenja, boba in čičerike, ki jih je napadla vrsta* O. crenata *na podlagi kvalitativnih in kvantitativnih parametrov. Avtorji so opazili veliko genetsko variabilnost v kvalitativnih znakih zajedavskih rastlin na intra- in inter-gostiteljskem nivoju. Opazili so razlike v steblu, listih, venčnih in cvetnih listih, čaši, osrednji brakteji cvetov, brazdi in prašnikih. V nasprotju so bile razlike na nivoju kvantitativnih znakov majhne. Variacije med zajedavskimi rastlinami glede na tip gostitelja so bile značilne le na nivoju obsega ogrlice in sesalnih korenin. Izsledki te študije bodo verjetno nadalje razloženi s specifičnimi odnosi med zajedavcem in gostiteljem ter prilagoditvami.*

Ključne besede: korenje, čičerika, bob, genetska variabilnost, morfološka primerjava, *Orobanche crenata*

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