HERITABILITIES AND GENETIC CORRELATIONS BETWEEN FLORAL AND REPRODUCTIVE TRAITS IN *Hedysarum coronarium* L. (FABACEAE)

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ABSTRACT

Six floral inflorescences, floral and reproductive characters from three cultivars and eight wild populations of an annual forage species *Hedysarum coronarium* L. were analysed. Only four traits : petal width, total number of inflorescences per plant, mean number of flowers per inflorescence and fruits proportion per flower, showed significant heritabilities between populations and cultivars. The fruit set trait exhibited the lowest significant heritability, suggesting a little genetic control and/or a greater environmental effect due to insect-pollinators, low density and activity. Multitrait genetic divergence among populations was assessed *via* principal components analysis carried out using a genetic correlation matrix. It appears that the cultivar "Grimaldi", (erected form), was genetically different from the prostrate form (Italian cultivars) and all the wild populations studied.

Keywords : *Hedysarum coronarium* L., genetic correlations, heritability, Tunisia.

RESUME

HERITABILITE ET CORRELATIONS GENETIQUES ENTRE DES CARACTÈRES FLORAUX ET REPRODUCTEURS ETUDIES CHEZ Hedysarum coronarium *L. (FABACEE)*

Six caractères floraux et reproductifs ont été étudiés chez trois cultivars et huit populations naturelles de l'espèce fourragère Hedysarum coronarium *L. Les héritabilités inter-populations de quatre caractères : largeur du pétale, nombre total d'inflorescences par plante, nombre moyen de fleurs par inflorescence et proportion de gousses par fleur, sont significativement différents. Ce dernier trait reproductif montre la plus faible valeur d'héritabilité, suggérant un contrôle génétique faible et/ou une forte action de l'environnement due à la faible densité et à l'activité des insectes pollinisateurs. La diversité génétique entre les populations a été évaluée au moyen de l'analyse en composantes principales effectuée sur la matrice des corrélations génétiques entre les variables mesurées. Il ressort que le cultivar « Grimaldi », de forme dressée, est génétiquement différent des cultivars Italiens prostrés ainsi que de toutes les populations naturelles étudiées.*

Mots clés *:* Hedysarum coronarium *L., corrélations génétiques, héritabilité, Tunisie.*

INTRODUCTION

Hedysarum coronarium L. (2n=2x = 16) is the most important forage legume species of the genus *Hedysarum* (Fabaceae). It is widespread in the Mediterranean basin, provides herbage of high nutritive value and contributes a considerable quantity of nitrogen to the soil.

Native plants of this annual taxon are predominantly prostrate, with short internodes and small leaflets. In natural popu-

lations, the frequency of individuals with erect growth habit is low (Figier *et al.*, 1978 ; Figier, 1982). The main objective of *H. coronarium* breeding programmes has been to improve agronomic characteristics, particularly to obtain erect varieties. The Italian cultivar "Grimaldi" was the most important one. This cultivated variety, recently introduced in Tunisia (North-Africa), exhibits a good biomass with a developed principal stem, long leaves and large leaflets.

However, the commercial success of a variety must also depend on its ability to produce reasonable quantities of seeds. Several factors may act on seed yield. In particular, breeding system and reproductive characters play a prominent part (Charlesworth, 1986 ; Morgan and Schoen, 1997).

Little is known about the fertility of the Italian cultivar "Grimaldi". In nature, pollen availability may be the most important ecological factor which significantly influences the fertility of the insect pollinating taxon. The native bee (*Apis mellifera*) is the main pollinator of *H. coronarium*. This species is adapted for entomophile pollination by several floral characteristics : large number of inflorescences, flowers per inflorescence, intense red coloured flowers, large carene and high pollen yields (Chriki *et al.*, 1984).

The flowers are self-compatible but protandrous, with earlier maturation of pollen than stigmas. Outcrossing rate, estimated by qualitative markers, based on floral coloration, is close to 90 % (Chriki *et al.*, 1984). Selfing may include autogamy (self–pollination within a flower), geitonogamy (insect-mediated pollination between flowers in the same inflorescence or within a plant) and bi-parental inbreeding (outcrossing between relatives) caused by limited seed dispersal and near-neighbor pollination.

In *H. coronarium*, the multi-ovulation fruit, resulting from an insect–pollinated flower, is a pod with 2-4 seeds. Sometimes, the pod contains one or more aborted seeds. The seed abortion may be an expression of inbreeding depression due to selfing.

This work, using wild and cultivated varieties of *H. coronarium*, is aimed at studying some of the ecological genetics of fertility in this taxon. In particular, the interpopulation genetic divergences for floral and reproductive traits were analyzed essentially by a multivariate approach. For predictions of the response to selection, the heritabilities of these traits were also estimated.

MATERIALS AND METHODS

REPRODUCTIVE BIOLOGY

H. coronarium usually flowers from late March until June. Flowers were pollinated by a suite of native bee species (especially *Apis mellifera*). Each mature plant produces 15-30 inflorescences (figure 1), each containing 20-40 flowers. The older flowers are beneath the inflorescences, each flower remaining receptive for about two days after anthesis. Fruits (pods) usually contain 2–4 articles per pod, each article containing one seed. The entire fruit (hence each article) is covered by very small thorns. Fruits were retained on plants until maturation in late July. Natural fruit-set is usually about 30-50 % of the flowers. Seed dispersal, via pod (or article) transport, is ensured by animals (particularly by Ovine). Seeds germinated upon the first autumnal rains.

PLANT COLLECTION

Up to one hundred seed samples were collected in native pastures as pods, from different natural sites in Tunisia, Algeria, Morocco, Italy and Sardinia between 1976 and 1983 (Baatout, 1995). Seeds from *H. coronarium* and from other species of the genus *Hedysarum* collected

Figure 1 : A picture showing an erect plant of *H. coronarium* at flowering stage.
This phenotype is rarely observed in natural populations, but it is a characteristic of the Italian cultivar "Grimaldi" recently introduced in Tunisia).

Une photo montrant une forme dressée de H. coronarium.
Ce phenotype est rarement observé dans la nature, mais caractéristique de la variété «Grimaldi» récemment introduite en Tunisie).

during these missions were conserved in the Laboratory of Genetics (Faculté des Sciences de Tunis). Seeds from Italian cultivars were added to the germplasm collection.

EXPERIMENTAL DESIGN

Eight wild populations from Tunisia, Algeria and Morocco, covering much of this natural range of habitats of *H. coronarium*, and three Italian cultivars were used in this study. Some of their agronomic traits are described in table 1.

Up to 20 mature fruits per maternal plant were collected from about 40 randomly chosen plants per population. Seeds were removed from pods and sown in Petri dishes on moistened whatman filter papers, and put at 20-25 °C in the dark in the laboratory. Seven days after sowing, one seedling, randomly selected from each maternal plant, was planted in each peat pot. At least 35 maternal plants were sampled per natural population. About 40 seeds randomly chosen from each Italian cultivar were planted under the same conditions. After two months, three seedlings randomly selected from each population were transplanted to bigger-size clay pots. These seedlings were placed in random blocks (one seedling per population per block) in the unheated greenhouse of the "Faculté des Sciences de Bizerte" (Tunisia), where they were cultivated and allowed to reach maturity.

Table 1 : Origin, principal agro-morphological and corresponding CVs traits and morphs of wild and cultivated populations of *H. coronarium* L.

Origine, principaux traits agromorphologiques et formes de populations sauvages et cultivées de H. coronarium *L.*

Cultivated population/size	Values of morphological traits and morph						
	Origin	LOS	(CV)	LHS	(CV)	LOS/LHS	Morph *
Mont (27)	Italy	2.43	(30)	81.20	(19)	0.03	Prostrate
Vici (26)	Italy	17.81	(42)	104.62	(21)	0.17	Mixed 1
Nad (29)	Tunisia	22.61	(87)	95.79	(18)	0.23	Mixed 1
Elal (30)	Tunisia	5.48	(90)	76.88	(22)	0.07	Prostrate
Tun (27)	Tunisia	3.62	(97)	60.42	(20)	0.06	Prostrate
Boug (27)	Tunisia	1.70	(91)	66.56	(19)	0.03	Prostrate
Al 56 (30)	Algeria	26.25	(75)	50.57	(22)	0.52	Mixed 2
Mak (30)	Tunisia	2.93	(83)	72.10	(27)	0.04	Prostrate
Grim (25)	Italy	94.34	(28)	37.56	(83)	2.51	Erect
Moro (30)	Morocco	27.90	(79)	88.60	(20)	0.31	Mixed 2
Sa004 (29)	Sardinia	15.00	(96)	94.34	(21)	0.16	Mixed 1

(*) At the individual level, a plant is prostrate if "LOS / LHS" is < 1/10.

At the population level, the following classes are defined :
- prostate, if the frequency of prostrate individuals (f p) is > 75 %
- erect, if " fp " < 25 %
- mixed 1(or predominantly prostrate), if "f p" is between 50 and 75 %
- mixed 2 (or predominantly erect), if " f p " is between 25 and 50 %.

LOS : mean height of principal stem at the end of plant development (cm) ;
LHS : mean length of the most developed horizontal branch (cm);
number of individuals (n) and coefficient of variation (CV) are in parentheses.

CHARACTERS MEASURED

When flowering began (March), a total of five flowers per plant was collected to determine petal width (PW; mm) at the widest point. The number of flowers per inflorescence (NFI) was estimated by counting the total number of flowers on five inflorescence per individual. At fructification (late April – May), the total number of inflorescences (NI) on each plant was counted.

The fertility was evaluated by two indices : the proportion of fertilized flowers (PF) and the (mean) number of seeds per fruit (SP). These parameters were determined on all flowers from five infrutescences per individual (late June – July). The proportion of aborted seeds (AS) was also estimated on the same infrutescence used for PF and SP. Because *H. coronarium* is predominantly outcrossing, a bee-hive was implanted in the greenhouse before flowering.

DATA ANALYSIS

Genetic and environmental components of variance and covariance were calculated, as in Humphreys (1989), with 10 degrees of freedom (d.f.) for between populations, 2 d.f. for between blocks and 20 d.f. for populations x blocks (used as the basic error term) between-populations (table 2).

Heritabilities (h^2) were calculated for each trait as follows :

$$h^2 = \frac{g}{g + e}$$

where "g" is the between populations genetic component and "e" is the populations environmental component (including interaction effects).

The 99 % confidence interval was calculated with a modified method of Wolff and Delden (1987), as :

$$\frac{1}{1 + \frac{nF_{\alpha}}{F_p - F_{\alpha}}} < h^2 < \frac{1}{1 + \frac{nF_{1-\alpha}}{F_p - F_{1-\alpha}}}$$

where $F_p = MS_P/MS_{PB}$, "F_a" is the critical value of the relevant F-distribution and "n" the number of populations.

Analysis of covariance were carried out on all pairs of floral and reproductive traits, and genetic correlations were calculated using the following models (Humphreys, 1989) (table 3).

Principal components analysis was carried out on phenotypic (p), genetic (g) and environmental (e) correlation matrices, using MINITAB statistical package (version 10.5). Principal components represent non correlated complex variables derived from the original correlated measured variables. The relationship between original and derived variables is indicated by the size and the sign of the principal component loading. Interpretation of component loading requires considerable caution although they can prove helpful in attempts to identify broad patterns of character relationships (Humphreys, 1989).

Table 2 : Analysis of variance model (populations fixed, blocks random).

Modèle d'analyse de variance (populations fixes, blocks randomisés).

Parameters	d.f.	Mean square Components
Populations (P)	10	$\sigma^2_E + \sigma^2_{PB} + 3.k^2_p$
Blocks (B)	2	$\sigma^2_E + 11\sigma^2_B$
PxB	20	$\sigma^2_E + \sigma^2_{PB}$

***Between-populations environmental component :**

$e = \sigma^2_E + \sigma^2_{PB}$ (PxB mean square)

***Between-populations genetic component :**

$g = k^2_p$ [(populations mean square – PxB mean square)/3]

Table 3 : Analysis of covariance model (for x and y) :

Modèle d'analyse de covariance (pour x et y)

Parameters	d.f.	Mean cross product Components
Populations (P)	10	$Cov_E + Cov_{PB} + 3\ k_x k_{y\ p}$
Blocks (B)	2	$Cov_E + 11\ Cov_B$
PxB	20	$Cov_E + Cov_{PB}$

***Environmental covariance :**

$Cov_E = Cov_E + Cov_P$ (PxB mean cross product)

***Genetic covariance :**

$Cov_g = K_x k_{y\ p}$ [(populations mean cross product - PxB mean cross product)/3]

***Genetic correlation :**

$$\frac{k_x k_{y\ p}}{\left(k^2_{x\ p} k^2_{y\ p}\right)^{1/2}}$$

***Environmental correlation :**

$$\frac{Cov_E + Cov_{PB}}{\left[(\sigma^2_E + \sigma^2_{PB})_x (\sigma^2_E + \sigma^2_{PB})_y\right]^{1/2}}$$

RESULTS AND DISCUSSION

UNIVARIATE ANALYSIS AND TRAIT HERITABILITIES

The Bartlett's test of homogeneity of variance among populations as well as the Anderson-darling's test of normality were not significant for most traits (table 4) ; so, original data were used without transformation.

Environmental and genetic components of variability from analysis of variance on non-standardized data for six measured traits are given in table 5. Of the six variables, only one -the mean number of seeds per fruit (SP)- failed to show significant difference between populations and was omitted from further analysis.

Heritabilities and corresponding 9 9 per cent intervals are also given in table 5. Except for the flowering characters : proportion of aborted seeds (AS) and mean number of seeds per fruit (SP), all other traits demonstrated significant heritabilities that were moderate (0.25) to high (0.89).

In our design, heritabilities measure the magnitude of the traits among populations of variation relative to the total phenotypic variation without inclusion of the variation due to block. Effects due to block were tested (results not shown) and found to be non-significant for most characters (except NFI and PF). So, the inclusion of the variation due to block did not significantly decrease heritabilities.

Table 4 : Bartlett's test of homoscedasticity (with respect to population factor) and Anderson-Darling's test of normality for six floral and reproductive traits of *H. coronarium.*

Test de Bartlett de l'homoscedasticité (selon le facteur population) et test de normalité de Anderson-Darling, de six traits floraux et reproductifs chez H. coronarium.

Reproductive traits	P- values	
	Bartlett's test	Anderson-Darling's test
NFI	0.342	0.108
NI	0.466	0.527
PW	0.373	0.576
PF	0.199	0.069
SP	0.015*	0.417
AS	0.616	0.149

(*) heritabilities not significantly different form zero (P>0.01).
NFI = number of flowers per inflorescence ; NI = number of inflorescence ; PW = petal width ;
PF = proportion of fertilized flowers ; SP = number of seed per fruit ; AS = proportion of aborted seeds.

Table 5 : Measured reproductive traits with associated environmental (e) and genetic (g) components of variability and level of significance (P) of g of *H. coronarium.*

Traits reproductifs mesurés associés aux composantes génétiques (g) et environnementaux (e) de la variabilité et niveau de signification (deg) chez H. coronarium.

Reproductive traits	Environmental and genetic components				
	e	g	p	h^2	(range)
NFI	13.1500	37.7600	0.000	0.74	(0.14-0.79)
NI	45.9800	66.9100	0.001	0.59	(0.05-0.67)
PW	0.0004	0.0032	0.000	0.89	(0.40-0.92)
PF	0.0045	0.0049	0.003	0.52	(0.02-0.62)
SP	0.0453	0.0149	0.092	0.25*	(-0.02-0.41)
AS	0.0029	0.0020	0.015	0.41*	(-0.01-0.53)

(*) heritabilities not significantly different form zero (P>0.01).
NFI = number of flowers per inflorescence ; NI = number of inflorescence ; PW = petal width ;
PF = proportion of fertilized flowers ; SP = number of seed per fruit ; AS = proportion of aborted seeds.

The floral trait (PW) and the major components of fecundity (e.g. number of inflorescence : NI and number of flowers per inflorescence : NFI) exhibited high heritabilities relative to fertility characters (PF, AS and SP). The heritability estimated here may include additive-genetic variation as well as variation due to dominance and epistatic relations between genes. Traits associated with low heritabilities, such as the fertility traits (PF, AS and SP), would be subject to a greater amount of environmental variations. For insect-pollinated species (like *H. coronarium*), fertility may be strongly affected by the density and activities of pollinators. In contrast, traits directly related to breeding parameters (e.g. flower size and flower number) are often associated with moderate or high heritabilities (Carr and Fenster, 1994).

Since correlations between two characters can be caused by environmental and/or genetic correlation, these two types of matrices were separately extracted from the phenotypic correlation matrix. Compared to genetic correlation (table 6), environmental correlation (not shown) between traits were generally small, thus tending to reduce phenotypic correlation (table 6). In an extreme case, an environmental correlations was significant and non-significant for a genetic correlation. For example, the genetic correlation between PW and AS was 0.079 while the environmental correlation was – 0.371.

2- The character such us number of flowers per inflorescence (NFI) was positively and significantly correlated with two other traits: petal width (PW) and proportion of fertilized flowers (PF). The character NFI, which indirectly determine seed yields, could be used as a measure of reproductive fitness.

3- The characters of fertility: proportion of fertilized flowers (PF) and proportion of aborted seeds (AS) showed the highest (negative) coefficient of genetic correlation (r = -0,735). This result clearly confirms that *H. coronarium* is predominantly out-crossing : a high cross-fertilization fruit set would contrast with a low seed abortion which can be related to selfing and considered as a manifestation of an inbreeding depression.

Table 6 : Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between the floral and reproductive traits studied in *H. coronarium.*

Correlations génétiques (sous la diagonale) et phénotypiques (au-dessus de la diagonale) des traits floraux et reproducteurs chez H. coronarium.

	NFI	NI	PW	PF	AS
NFI	1.000	- 0.656	0.482	0.357	- 0.077
NI	- 0.378	1.000	- 0.496	0.213	- 0.143
PW	0.406	- 0.345	1.000	0.101	0.079
PF	0.155	0.226	0.024	1.000	- 0.735
AS	- 0.282	- 0.159	- 0.026	- 0.089	1.000

Degrees of freedom = 31 ; Lovel of significante : 0.344 (p=5 %) ; 0.443 (p=1 %).
(*) heritabilities not significantly different form zero (P>0.01).
NFI = number of flowers per inflorescence ; NI = number of inflorescence ; PW = petal width ;
PF = proportion of fertilized flowers ; SP = number of seed per fruit ; AS = proportion of aborted seeds.

The main points of interest between characters in genetic and phenotypic correlations (table 6) include :

1- The most important components of fecundity (e.g. the number of inflorescence: NI and the number of flowers per inflorescence : NFI) showed a high negative coefficient of genetic correlation (r = - 0.656). A little production of inflorescence may be compensated by a greater number of flowers per inflorescence.

MULTIVARIATE ANALYSIS

Principal Component analysis was carried out on phenotypic, genetic and environmental variance/covariance matrices derived from data for five traits. The cumulative percentage variance accounted for by the latent root of the first three principal components was analyzed (table 7).

Table 7 : Cumulative percentage variance (and variance) accounted for by successive principal components derived from phenotypic (p), genetic (g) and environmental (e) variance/covariance matrices for five reproductive traits of *H. coronarium*.

Variance cumulative (%) (et variance) résultant des composantes principales issues des matrices variance/covariance phenotypiques (p) génétiques (g) et environnementaux de cinq traits chez H. coronarium.

	Cumulative percentage variance according to component number		
Matrix	1	2	3
p	35.3 (1.766)	61.8 (1.324)	80.1 (0.915)
g	42.3 (2.113)	79.0 (1.834)	90.5 (0.573)
e	35.2 (1.762)	63.8 (1.429)	83.7 (0.995)

It is clear that in the analysis of the genetic matrix, at least 90 % of the variance were accounted for by the first three principal components while those derived from the analysis of phenotypic and environmental matrices were about 80 % and 84 %, respectively. This reflects stronger correlations between traits when based on genetic than on environmental effects, and suggests an underlying genetic relationship between traits. Interactions with the environment were more independent between traits and tended to mask genetic relationships, as shown by the relatively lower variance accounted for by components derived from the phenotypic matrix.

Genetic correlation may arise from pleiotropy or linkage disequilibrium or both (Prosperi *et al.*, 1995). As they determine how traits will change in relation to each other, an understanding of genetic correlation among life history traits is crucial to an understanding of coordinates evolution through correlated responses to natural selection (Falconer, 1981). In the context of plant breeding, a certain comprehension of genetic correlation is particularly useful in performing indirect artificial selection on characters that show low heritabilities.

The relative contribution of each of the traits to the variance retained by the principal components and estimates of heritabilities for the traits were analyzed (table 8). Correlation between the heritability estimates and the communalities from the phenotypic, genetic and environmental matrices were –0.92, 0.68 and 0.41, respectively.

Communalities indicate the relative weights of traits in the total variance accounted for by the three principal components. The genetic matrix produced weights which were more positively (but not significantly) related to the heritability of traits than those produced by the environmental matrix. Weights produced by the phenotypic matrix had a strong negative relationship with heritability. Thus there were clear advantages to removing environmental effects from the phenotypic correlation matrix. Godshalk and Timothy (1988) suggested that the extraction of genetic matrices had little effects on rankings for principal components scores obtained from simple phenotypic matrix based on a limited range of traits.

Table 8 : Communalities of five traits based on the first three principal components derived from phenotypic (p), genetic (g) and environmental (e) variance/covariance matrices and reproductive trait heritabilities of *H. coronarium*.

Communalités de cinq traits basées sur les trois composantes principales issues de matrices variance/covariance phénotypiques (p), génétiques (g) et environnementales (e) et leur héritabilité chez H. coronarium.

Reproductive traits	Communalities			Heritabilities
	p	g	e	
NFI	0.76	0.87	0.80	0.74
NI	0.75	0.91	0.74	0.59
PW	0.61	0.98	0.96	0.89
PF	0.94	0.93	0.80	0.52
AS	0.94	0.83	0.88	0.41

NFI = number of flowers per inflorescence ; NI = number of inflorescence ;
PW = petal width ; PF = proportion of fertilized flowers ; AS = proportion of aborted seeds.

However, principal components analysis carried out on genetic matrix derived from data for only five traits appear to have some advantages. Virtually, all the variation in the extracted genetic matrix was summarized in three principal components and the relative contributions of traits to these was directly related to their heritabilities.

Principal components analysis can also be used in multivariate selection programmes (Godshalk and Timothy, 1988). The relative contribution of traits to each principal component (component loading) can aid in the interpretation of the relationships between traits and identify combinations amenable to independent selections. Loading for the first three principal components extracted from the genetic matrix has been studied (table 9). It appears that the first component essentially reflects the fecundity of plants. It clearly opposes to the character total number of inflorescences (NI) to the character number of flowers per inflorescence (NFI). The second component is associated with the fertility performances of individuals. It is strongly and positively correlated with the proportion of aborted seeds (AS) and negatively correlated with the percentage of fertilized flowers (PF). The third component is especially associated with the size of flowers (PW).

To assess the overall genetic diversity among wild and cultivated populations of *H. coronarium* used in the current study, the principal components loading and standardised population means for each trait were used to generate population scores. Essentially, these provided a summary of the genetic composition of each population in terms of traits relevant to breeding objectives.

The scatter plot of population scores for the first two principal components (figure 2) derived from the genetic variance/covariance matrix of five traits showed four groups. Three of the five reproductive traits were formed with respect to the second principal component. Their "vertical" repartition would be determined by the fertility characters strongly corrected with this second principal component. The fourth group, containing the only erect cultivar ("Grimaldi"), was strongly and

positively correlated with the first principal component. This Italian cultivar actually exhibited the highest (NFI) and the lowest (NI) (table 10).

Table 9 : Loading for the first three principal components derived from the genetic correlation matrix for five reproductive traits of *H. coronarium*.

Contribution des cinq traits selon les trois premières composantes principales issues des matrices de correlation chez H. coronarium.

Reproductive trait	Correlations according principal components		
	1	2	3
NFI	0.894	- 0.123	0.244
NI	- 0.814	- 0.366	- 0.332
PW	0.767	0.120	- 0.616
PF	0.242	- 0.926	- 0.096
AS	- 0.062	0.902	- 0.118

NFI = number of flowers per inflorescence ;
NI = number of inflorescence ; PW = petal width ;
PF = proportion of fertilized flowers ;
SP = number of seed per fruit ;
AS = proportion of aborted seeds.

The unambiguous separation of the Italian cultivar "Grimaldi" from all other populations of *H. coronarium* may be related to its erect morphology (morph). To test this hypothesis, a discriminate analysis was performed on non transformed data derived from reproductive five traits. The variable "morph" (table 1) was used as a classification factor. Mahalanobis distances representing the extent of genetic separation between groups were studied (table 11). The percentage of individuals correctly classified into their corresponding groups was important (81,8 %), indicating a net genetic divergence between morphs.

The strong genetic variation between classes, in particular the separation of the erect morph from all other groups, may be used to determine which inter-morph crosses are likely to give most heterosis through genetic recombination. For the successfull realization of this breeding program, a more precise knowledge of the mating behaviour of *H. coronarium*

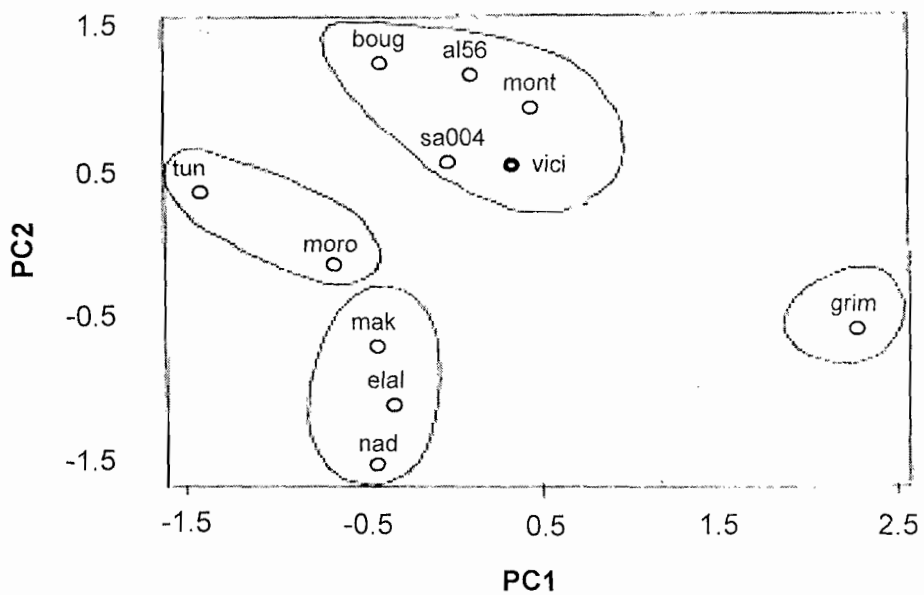

Figure 2 : Scatter plot of population scores for the first two principal components (PC1 and PC2) from a genetic variance/covariance matrix of five traits showing groups formed of *H. coronarium*.

Répartition des populations selon les deux premières composantes principales (PC1 et PC2) à partir d'une matrice génétique variance/covariance, des groupes de cinq traits formés chez H. coronarium.

would be helpful. So, mating parameters of this allogamous species have been recently estimated (Yagoubi and Chriki, 2000).

In conclusion, genetic variability assessment of the annual allogamous species, examined with respect to floral and reproductive characters, showed that natural populations are morphologically distinguished from erect Italian cultivars recently introduced in Tunisia. However, it is well known that the study of the distribution of a given species and the identification of the main characteristics responsible for this distribution are the basis of the understanding of the evolution of biological diversity. Because morphological traits only represent a part of the genetic diversity, and because the distribution of this diversity is highly determined by natural selection, the identification of the mechanisms involved within populations or species (gene flow, breeding characteristics, etc.) requires some studies on neutral genetic variability (i.e. not submitted to natural selection). In the context, several genetic studies based on neutral markers such as allozymes or DNA analysis may be the future goal to specify the genetic structure of natural and cultivated populations of *H. coronarium*.

Table 10 : Population means of floral, fecundity and fertility traits studied in *H. coronarium*.

Populations moyennes pour les traits floraux, de fertilité et de fécondité étudiés chez H. coronarium.

cultivated/ population (age)	NFI	NI	PW	PF	AS
Mont (3)	30.25 (0.71)	17.15 (3.72)	0.67 (0.01)	0.33 (0.06)	0.10 (0.09)
Vici (3)	35.86 (2.77)	30.37 (6.07)	0.70 (0.01)	0.43 (0.11)	0.14 (0.03)
Nad (3)	30.95 (7.49)	45.20 (13.34)	0.65 (0.01)	0.56 (0.01)	0.06 (0.04)
Etal (3)	24.78 (4.04)	29.10 (5.46)	0.63 (0.03)	0.54 (0.09)	0.05 (0.01)
Tun (3)	22.08 (2.18)	39.20 (4.61)	0.59 (0.01)	0.42 (0.16)	0.16 (0.06)
Boug (3)	26.38 (1.88)	26.23 (2.11)	0.62 (0.01)	0.41 (0.01)	022 (0.05)
Al 56 (3)	22.72 (6.17)	30.33 (10.06)	0.78 (0.01)	0.41 (0.11)	0.19 (0.05)
Mak (3)	22.66 (4.20)	37.23 (3.75)	0.69 (0.03)	0.51 (0.09)	0.07 (0.08)
Grin (3)	43.26 (7.39)	15.88 (2.99)	0.76 (0.02)	0.59 (0.06)	0.11 (0.07)
Moro (3)	25.42 (3.17)	37.47 (5.83)	0.64 (0.02)	0.48 (0.12)	0.13 (0.06)
Sa 004 (3)	30.46 (4.94)	25.51 (4.69)	0.70 (0.01)	0.39 (0.07)	0.10 (0.02)

(standard deviations are in parentheses); **n** is the number of individuals per population.

Table 11 : Mahalanobis distances between the four groups identified by their morphs in *H. coronarium.*

Distances de Mahalanobis entre les quatre groupes identifiés par leurs formes chez H. coronarium.

Morph	1	2	3	4
1	0.00			
2	4.54	0.00		
3	4.17	3.91	0.00	
4	28.38	15.43	26.22	0.00

1 : prostrate ; 2 : predominantly prostrate ; 3 : predominantly erect ; 4 : erect.

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