Autogamy and allogamy in genus *Lathyrus*

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Introduction

In Tunisia the genus *Lathyrus* is represented by 15 native species [(11)], distributed essentially in marginal environments in the north of the country. Many of them are of considerable agronomic interest as forage (*L. ochrus* and *L. articulatus*) and as human food (*L. cicera* and *L. sativus*). These species are under utilised and in the past little effort has been made to expand the selection and breeding of *Lathyrus* as a food crop. However, many *Lathyrus* species have potential as an alternative pulse in many cropping systems as they are tolerant to low rainfall conditions. Research is now being conducted to address aspects of improvement of some species such as *L. sativus*, including reducing concentrations of the neurotoxin β-N-oxalyl-L-α, β-diaminopropanoic acid (ODAP) in the seed, introducing insect and disease resistance, and increasing yield [(4)].

In order to identify useful material for agriculture and for breeding programs, we studied breeding systems and analysed some aspects of floral biology of fourteen annual and perennial *Lathyrus* species.

Materials and methods

Fourteen annual and perennial *Lathyrus* species belonging to four sections according to Kupicha's classification [(10)] were analysed, each species was represented by one population. All these species are diploid (2n=2x=14). Studied species, their biological type and their sites of origin are listed in Table 1, only *L. sativus*, *L. cicera* and *L. odoratus* (the ornamental sweetpea) are cultivated.

The seeds used in this study were germinated in petri dishes on wet filter paper. They were manually scarified in order to increase their germination percentage. Seedlings were transplanted in October to pots, 20 cm in diameter, the plants were grown under homogenous conditions (25°C and 12h /day length) in an experimental garden, at the National Agronomic Research Institute of Tunisia (Upper Semi-arid). Plant flowering occurred from April (*L. cicera*, *L. sativus* and *L. aphaca*) to June (*L. latifolius* and *L. sylvestris*).

Three types of treatments were performed: selfing by bagging flowers in order to prevent insect visitation of the flower (Treatment A), natural pollination without castration of anthers (Treatment B) and natural pollination following castration of anthers (Treatment C). Each type of operation was performed on 15 flowers per plant, 5 plants were studied per species. Two fertility parameters were measured for each plant on 15 flowers per plant: the frequency of flowers giving pods (*p/f*) and the mean number of seeds per pod (*g/p*).

The values of the ratio *p/f* ranged from 0 to 1, the transformation

$f(x) = \arcsin\sqrt{x}$, where $x = p/f$, was used.

Statistical analysis. The data of the fertility parameters were analysed by analysis of variance to test differences between species and between species and breeding system effects. The Duncan test [(7)] was used to compare means. The SAS procedure ANOVA was used.

Results

Selfing by bagging flowers. The three perennial species *L. latifolius*, *L. sylvestris* and *L. tuberosus* did not develop pods by selfing, the ratios *p/f* and *g/p* were both zero (Table 2) and indicate that these species are strictly outcrossing. For that reason they were excluded from statistical analysis. The variance analysis showed highly significant differences between annual species for the two studied fertility parameters: number of flowers giving pods *p/f* (F ratio 6.04) and number of seeds per pod *g/p* (F ratio 37.41).

The comparison of means using Duncan test at 5 % (Table 3) reveals a regrouping of most species for high means for the number of flowers giving pods (*p/f*). However, *L. odoratus* is distinguished from other species by a low mean for *p/f* (0.25) (Table 2).

The comparison of means at 5% showed that *L. nissolia* had the highest number of seeds per pod (9.30) (Tables 2 and 4). This can be explained partly by the high number of ovules per ovary in this species (Table 5). *L. aphaca*, *L. cicera*, *L. sativus* and *L. setifolius* had the lowest means for *g/p* (range 2.44 to 4.12).

Table 1. *Lathyrus* species studied, nomenclature of Kupicha[(10)].

Species	Abbreviation	Section	Habit	Origin
L. annuus	AN	Lathyrus	Annual	Tunisia
L. aphaca	A	Aphaca	Annual	Italy
L. articulatus	AR	Clymenum	Annual	Tunisia
L. cicera	C	Lathyrus	Annual	Tunisia
L. hirsutus	H	Lathyrus	Annual	Italy
L. latifolius	L	Lathyrus	Perennial	Tunisia
L. nissolia	N	Nissolia	Annual	France
L. odoratus	O	Lathyrus	Annual	Tunisia
L. ochrus	OC	Clymenum	Annual	Tunisia
L. sativus	SA	Lathyrus	Annual	Tunisia
L. setifolius	SE	Lathyrus	Annual	Italy
L. sylvestris	S	Lathyrus	Perennial	France
L. tingitanus	TI	Lathyrus	Annual	Portugal
L. tuberosus	T	Lathyrus	Perennial	Hungary

Table 2. Frequency of flowers giving pods and mean number of seeds per pods in selfing and in natural pollination.

Species		Frequency of flowers giving pods (*p/f*)			Mean number of seeds per pod (*g/p*)		
		A	**B**	**C**	**A**	**B**	**C**
L. annuus	AN	0.82	0.84	0	5.33	5.43	0
L. aphaca	A	0.72	0.79	0	4.12	4.32	0
L. articulatus	AR	0.64	0.88	0	5.31	5.38	0
L. cicera	C	0.91	0.86	0.13	3.58	3.44	3
L. hirsutus	H	0.88	0.90	0	5.80	6.25	0
L. latifolius	L	0	0.85	0.11	0	6.34	6.37
L. nissolia	N	0.75	0.82	0	9.30	9.75	0
L. ochrus	OC	0.81	0.80	0	5.11	4.63	0
L. odoratus	O	0.25	0.81	0.20	5.84	6.67	5.46
L. sativus	SA	0.53	0.80	0.09	2.82	2.50	2.57
L. setifolius	SE	0.66	0.72	0	2.44	2.70	0
L. sylvestris	S	0	0.73	0.13	0.20	5.07	6.40
L. tingitanus	TI	0.68	0.89	0	6.33	6.70	0
L. tuberosus	T	0	0.88	0	0	4	0

A selfing by bagging
B natural pollination without castration
C natural pollination following castration

Natural pollination without castration of anthers. Variance analysis indicated no significant differences for the ratio *p/f* (F ratio1.56) and highly significant differences for fertility parameter *g/p* (F ratio 48.35).

The comparison of means by Duncan test at 5 % (Table 4) demonstrated a regrouping between all species for the number of pods per flower (*p/f*) (Table 3). However, *L. nissolia* is characterized by high values of *g/p*. *L. setifolius* and *L. sativus* have the lowest ratio of *g/p* and the lowest number of ovules per ovary (Table 5).

In natural pollination without castration of anthers, the values of the two fertility parameters are higher than those observed in selfing with bagging anthers (Table 2). This is particularly prominent for the three

perennial species *L. latifolius, L. sylvestris* and *L. tuberosus* , and also *L. odoratus*, all of which did not develop pods in selfing. The values of *p/f* increase respectively from 0 to 0.88 for *L. tuberosus*, from 0 to 0.85 for *L. latifolius*, from 0 to 0.73 for *L. sylvestris* and from 0.25 to 0.81 for *L. odoratus*.

Natural pollination following castration of anthers. The results of natural pollination following castration revealed that the castrated flowers from annual species (*L. aphaca, L. nissolia, L. articulatus, L. ochrus, L. tingitanus*, *L. annuus*, *L. setifolius* and *L. hirsutus*) did not develop pods and seeds (Table 2). However, for five species (*L. odoratus, L. sativus, L. latifolius, L. sylvestris* and *L. cicera*) the ablation of anthers caused a clear reduction in the number, but did not completely inhibit the development of pods and seeds. This indicates clearly that the three cultivated annual species (*L. odoratus, L. sativus* and *L. cicera*) can be pollinated either by their own pollen or by foreign pollen.

Variance analysis revealed no significant differences between the five species for the parameter *p/f* (F ratio 2.58) (Table 3) and highly significant differences for *g/p* (F ratio 12.83) (Table 4). The comparison of the means shows that *L. latifolius, L. sylvestris* and *L. odoratus* have the highest means for the number of seeds per pod (*g/p*).

It is important to mention that the values obtained by the analysed parameters are lower in natural pollination with castration than in natural pollination without castration. Some of this reduction could be attributed to lesion caused by castration.

Comparison of species by breeding system effects. Variance analysis with two classification criteria indicated that there is a species by breeding system effect, which is highly significant for the two studied parameters (F ratio 9.75, for *p/f* and F ratio 47.23 for *g/p*).

The comparison of averages reflects a regrouping for species for the parameter *p/f*. However, a heterogeneity is observed for the ratio *g/p*: *L. nissolia* is characterised by the highest means, while *L. setifolius* and *L. sativus* have the lowest means (Table 4).

Comparison between selfing with bagging, natural pollination without castration of anthers and natural pollination following castration of anthers. The comparison of the three breeding systems using Duncan test at 5 % reveals a separation of breeding systems for the two parameters *p/f* and *g/p*. For the ratio *g/p*, the two breeding systems: natural pollination and selfing by bagging are grouped with the highest means. Also, we observed a classification of the three breeding systems in the following decreasing order of efficacy: natural pollination without castration of anthers **B**, selfing by bagging flowers **A**, natural pollination following castration **C**. This occurred for both *p/f* and *g/p*.

Table 3. Comparison of means using Duncan test at 5% for the fertility parameter *p/f*. Treatments are selfing by bagging flowers (Treatment A), natural pollination without castration of anthers (Treatment B) and natural pollination following castration of anthers (Treatment C). Species are ranged in order from least to greatest, left to right. Species under a continuous bar are not significantly different to each other. Species abbreviations are found in Table 1.

A		C	H	AN	OC	N	A	TI	SE	AR	SA	O	
B	H	TI	T	AR	S	C	L	AN	N	O	OC	SA	A	SE
C		SA	L	S	C	O							

Comparing species across all of Treatments A, B and C:

T	H	AN	OC	C	A	N	AR	TI	SE	SA	S	L	O

Comparing Treatments A, B and C:

A	B	C

Table 4. Comparison of means using Duncan test at 5% for the fertility parameter *g/p*. Notation as for Table 3.

A		N	TI	O	H	AN	AR	OC		A	C	SA	SE	
B	N	TI	O	L	H	AN	AR	S	OC	A	T	C	SE	SA
C		S	L	O		C	SA							

Comparing species across all of Treatments A, B and C:

D	N	TI	O	H	L	OC	AN	AR	S	A	T	C	SE	SA

Comparing Treatments A, B and C:

E	B	A	C

Table 5. Means comparison using Duncan test at 5% for the parameter mean number of ovules per ovary (No/OV). Notation as for Table 3.

Species	L	N	S	T	O	TI	AN	OC	A	H	AR	C	SA	SE
No/OV	18.6	12.2	11.8	11.5	11.3	8.8	8.0	7.5	6.6	6.4	5.8	5.3	3.5	2.9

Table 6. Means comparison using Duncan test at 5% for the floral parameters: mean flower length in mm (LF) and mean number of flowers per inflorescence (NF/IN). Notation as for Table 3.

Species	O	L	TI	OC	T	S	AR	SA	N	C	SE	H	A	AN
LF	33.5	26.1	23.4	17.5	15.6	15.6	15.6	14.1	13.6	12.0	11.8	11.2	11.1	11.0

Species	L	S	T	O	TI	AR	H	SA	C	A	N	OC	SE	AN
NF/IN	10	7	3	3	2	2	2	1	1	1	1	1	1	1

Discussion

In selfing by bagging flowers, with the exception of *L. odoratus* (*p/f* = 0.25), the values of *p/f* are high in annual species and zero in the three perennial ones. The values of *p/f* in the ten annual species ranged from 0.53 (*L. sativus*) to 0.91 (*L. cicera*) and indicate that these species are preferentially autogamous [3,9,12].

The autogamous breeding system, which is a characteristic for annual species of genus *Lathyrus*, seems to be favored by certain characters related to the floral biology of plants. The comparison of percentage of selfing in ten annual species with means of the floral parameters (Table 6) measured on 60 flowers and with the flower colour show a relationship between the selfing ratio and these parameters. In fact, *L. cicera, L. hirsutus, L. annuus, L. ochrus, L. aphaca, L. nissolia, L. sativus, L. tingitanus, L. setifolius* and *L. articulatus* are characterised by a reduced number of flowers (1 to 2) per inflorescence (Table 6), flowers of small size (range mean 11 mm in *L. annuus* to 23.4 mm in *L. tingitanus*), a pale or dark flower colour (white or blue in *L. sativus*; yellow in *L. annuus, L. aphaca* and *L. ochrus*; blue in *L. hirsutus*; red in *L. cicera, L. setifolius* and *L. nissolia*). These parameters make these flowers less attractive to pollinators [6,13,15,17]. All these species have percentages of selfing higher (ranging from 0.53 in *L. sativus* to 0.91 in *L. cicera*) than those observed in *L. odoratus* (0.25) and in the three perennial species *L. latifolius, L. sylvestris* and *L. tuberosus* (0).

The lowering of fertility observed in selfing in all species could be explained by the stress on flowers due to bagging. The bagging of flowers increases the temperature and causes the fall of pollinated flowers before the maturity of pods. Similar observations have been made in the genus *Medicago* [5].

The high values of the ratio *p/f* obtained in natural pollination without castration express that the three perennial species are strictly outcrossing and *L. odoratus* is preferentially outcrossing [14]. The development of pods and seeds in perennial species is due to pollinators, especially bees and bumblebees. The flowers of *L. latifolius* are visited by many insects the same day; such as Coleoptera (*Bruchus* beetles), Hymenoptera (*Apis*, *Bombus* and *Xylocopidae*) and Lepidoptera [16]. In fact, the stigma of these species becomes receptive only after the rupture of the cuticle of the stigma papillae [1]. Reproductive characters maintained by natural selection allow these allogamous species to be adapted to entomophilic pollination. Flowers of large size, bright flower colour (red in *L. latifolius* and *L. sylvestris*, pink in *L. tuberosus*) flower density (an average of 10 flowers per raceme in *L. latifolius*, 7 in *L. sylvestris* and 3 in *L. tuberosus*) and nectar production [8].

In natural pollination following a castration, ablation of anthers leaded to the absence of developing pods in annual species (*L. aphaca*, *L.* nissolia, *L. articulatus*, *L. ochrus*, *L. tingitanus*, *L. annuus*, *L. setifolius* and *L. hirsutus*) and the perennial *L. tuberosus*. For annual species, this result shows that pollination is assured by their own pollen. For the three cultivated species (*L. odoratus*, *L. sativus* and *L. cicera*) ablation of anthers caused a great reduction of the fructification but did not inhibit it. This indicates that these species are pollinated by their own pollen and can receive foreign pollen. The absence or the reduction of fructification indicates that the pollen takes part in pollinator attraction, particularly bees [2].

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