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Floral Structure, Stigma Receptivity and Pollen Viability in Relation to Protandry in Motherwort (*Leonurus cardiaca* L.)

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ABSTRACT: *Leonurus cardiaca*, commonly known as motherwort, is a member of the Lamiaceae family. It has been used in the traditional medicine against nervous and functional cardiac disorders since the 15th century and now is described in pharmacopoeias for producing sedative, hypotensive and cardiotonic pharmacological effects. This experiment was accordingly conducted to investigate the flower structure, flower biology, pollination system, and breeding system. Autogamy, apomixy, xenogamy, and open pollination were examined by Cruden's method. The results show that *L. cardiaca* flowers are hermaphrodite and short-lived, which are arranged in raceme-like cyme inflorescences. Protandry is the dominant form, and the stigma reaches its most receptivity 48 hours after anthesis, while the highest in-vitro pollen germination is observed within two hours after anthesis. The style continues to growth after anthesis, reaching its maximum length 48 hours after flower opening, coinciding with the separation of stigma branches. Based on our results, *L. cardiaca*, is a 'facultative out crossing' plant. P/O level and pollen production were sufficient for the pollination mechanism that needs a large delivery of pollens to ensure an effective pollen deposition.

Keywords: Facultative outcrossing, pollination, protandry, Lamiaceae family

INTRODUCTION

The reproductive biology of flowering plants is important to determine barriers to seed and fruit set, to conserve them, and to understand the pollination and breeding systems that regulate the genetic structure of populations (Tandon *et al.,* 2003, Barrett, 2010). The timing of flowering can strongly influence the reproductive success of a plant in several ways (Rathckeand Lacey, 1985). Such effects may be mediated by abiotic factors and factors operating within plants, within populations, and between species (McIntosh, 2002).Variation in sexual plant breeding systems ranges from cleistogamy and in-bud pollination (mechanisms to ensure self-pollination) to dioecy and self-incompatibility (mechanisms that support cross-pollination). The pollen-ovule (P/O) ratio is an important floral trait that reflects the mating system of aplant (Cruden, 1977; Bennett, 1999; Jurgens *et al.,* 2002). Cruden (1977) observed that out breeding plants tend to have higher P/O ratios. He explained this finding by the 'efficiency of pollination': self-pollinating plants (*i.e.* autogamous mating system) need less pollen grains for an efficient pollination than those dependent on agents such as wind or animals (i.e. xenogamous mating system).

Lamiaceae is a large family, ca. 220 genera and 4000 species, with a cosmopolitan distribution, which is particularly well represented in warm temperate regions (Cronquist, 1981; Hedge 1992). Their peculiar floral structure calls for intricate pollination mechanisms, which reflects a long history of adaptive coevolution between the plants and their pollinators (Huck, 1992). Allogamy is a usual reproductive process in the plants. There is still no wholly convincing evidence that self-incompatibility occurs in every species in Lamiaceae. Protandry is common, and self-pollination is therefore almost impossible. Geitenogamy and cross-pollination are dominant in the family (Hidalgo and Ubera, 2001; Rodriguez-Riano and Dafni, 2007).

The reproductive biology of several species of Lamiaceae has been widely studied; for example, *Origanum majorana* and *Teucrium capitatum* (Rodriguez-Riano and Dafni, 2007), *Rosmarinus officinalis* (Hidalgo and Ubera, 2001), *S. smyrneae* (Subashi and Guvensen, 2011), and *Monarda fistulosa* (Cruden *et al.,* 1984). However, little is known about the genus *Leonurus*. Yeo *et al.* (2006) described flower and inflorescence developments of *L. sibiricus* and reported natural self-pollination and artificial cross-pollination. *Leonurus cardiaca* (Lamiaceae), commonly known as motherwort, is a perennial herb widespread in Europe, East Asia to the Himalayas, West Asia, Northern Africa, and North America. It is usually found in country areas throughout the hills and plains (Wojtyniak *et al.,* 2013). *L. cardiaca* is an important medicinal plant, growing in many regions of Iran. It has been used to cure cardiovascular diseases, stress, anxiety, and nervous irritability (Russian Pharmacopoeia, 1968; Milkowska-Leyck *et al.,* 2002). Its chemical compounds such as alkaloids, iridoids, flavonoids, saponins, cardenolid-like glycosides, and diterpenoids have been isolated from the leaves and flowers (Milkowska-Leyck *et al.,* 2002). Although there have been considerable studies of genetic diversity of *Leonurus cardiaca* as a medicinal plant, little is known about its reproductive biology. The present work on *L. cardiaca* covers: (a) the structural and temporal details of flowers, (b) pollination biology, and (c) the breeding system.

MATERIALS AND METHODS

A. Experimental site and plant materials

The present research was carried out at Research Center for Department of Horticulture, University of Tehran, Karaj, Iran, during 2015-2016 (36°19'0"N / 50°59'29"E, 1320 m a.s.l.). This area is a mountainous region with a cold and wet winter and a mild summer. The present experiment was conducted on 80 random plants (three-years old) originated from seeds collected from their natural habitat in Isfahan. The soil texture was clay loam.

B. Floral characters and phenology

The flowers were morphologically assessed for the trait slength of flower, filament, anther, upper lip, lower lip, and style, and corolla mouth diameter. Since the style length differs as the flower ages, it was measured at four different times: 2, 24, 48, and 72 h after anthesis. This experiment was arranged in a completely randomized design with 5 replications. Flower longevity was assessed in flowers in natural conditions, which were exposed to pollinators throughout anthesis. Twenty flowers were selected randomly and tagged. The flowers were visited daily until senescence, and the occurrence time of each of the following phenomena was recorded: anthesis, anther dehiscence, stigma lobes opening, senescence, and flower falling off. The anthers were hit gently with the tip of a glass rod to examine the dehiscence of anthers and pollen releasing. A hand magnifying lens was used to achieve more accurate findings.

In order to investigate the phenology of the species, some growth and developmental traits of reproductive stages (including flower emergence, flowering peak, end of flowering time, and seed maturation time) were recorded by repeated observations during the plant growth.

C. Pollen-ovule levels

Anthers were collected before anthesis, placed on a drop of distilled water on a microscopic slide, and squashed to release the pollens, and then the number of pollens per anther was counted (Bennett, 2001). The number of pollens per flower was calculated by the number of pollens per anther multiplied by 4, since there are four stamens. The number of ovules per flower was counted by a careful examination of flowers, and the ratio of the number of pollen grains to ovules was calculated and repeated for 15 flowers.

D. Pollen germination and stigma receptivity

To evaluate pollen germination at different stages of flower development, pollens were collected at four developmental stages, including 2, 24, 48, and 72 h after anthesis. To determine the germination, pollen grains were sowed with a clean brush in petri dishes containing culture medium (15% sucrose, 100 ppm boric acid (H_3BO_3) and 1% agar) according to Dane's *et al.* (2004) method. The petri dishes were incubated at the constant temperature of 25°C under usual light conditions (500 lux) for 24 h. The pollen grains were considered germinated when the pollen tube length was greater than the diameter of the pollen grain. A minimum of 100-150 pollens were counted per petri dishes with three replicates. Germination percentage was determined by dividing the number of germinated pollen grains by the total number of pollen per field of view. A light microscope was used to determine the pollen germination.

Timing of stigma receptivity was investigated by assessing seed set following controlled pollination.35 flowers were emasculated early in the morning before anthesis. Hand pollination was performed with freshly collected pollens at three different times: 24, 48, and 72 h after flower opening. Flowers were bagged and left until seed production.

E. Statistical analyses

Pearson correlation coefficients were calculated to investigate the relationships between the style length, stigma reception, and pollen germination. Analysis of variance was performed by One-way ANOVA using SPSS v.22 software (IBM Corp., 2013), and means were compared according to Duncan's multiple range test.

RESULTS

A. Floral characters and phenology

Flowers of *Leonurus cardiaca* are arranged in whorl clusters of 13-16 flowers in the angle of opposite leaves of uppermost part of the main and lateral stems. Flowers are zygomorphic and protandrous, which have a yellow to green calyx, a pink to white corolla with dark pink spots, four stamens with pale to dark yellow anthers, a superior ovary with four locules, four ovules in each ovary, and dichotomous styles. The fruit is a four trigonal achene (Fig. 1 (A and B)).

The corolla has well-developed upper and lower lips. Stamens are oriented towards the upper lip and located among the villous crown. Variation in quantitative characteristics of the flowers is shown in Table 1. The examination of the style length reveals that it increases gradually after anthesis and reaches its maximum length on the third day after anthesis. There was no significant difference between the third and fourth day after flower opening ($P < 0.01$; table 2).

Table 1: Floral characteristics (mean ± S.E) in the flower of *Leonurus cardiaca.*

Floral characteristics	length (mm)
Flower	10.53 ± 0.19
Filament	6.95 ± 0.19
upper lip	3.27 ± 0.14
lower lip	2.64 ± 0.11
Sepal	5.87 ± 0.20
Anther	0.74 ± 0.03
Corolla	8.07 ± 0.17
Diameter of corolla	1.15 ± 0.09

Table 2: Style length (mean ± S.E) in the flowers of *Leonurus cardiaca* at different floral age. means were compared by a Duncan's Multiple Range Test (P < 0.01).

Floral age	Style length (mm)
2 h after anthesis	6.56 ± 0.010^{a}
24 h after anthesis	7.48 ± 0.015^{b}
48 h after anthesis	8.12 ± 0.011^{c}
72 h after anthesis	8.13 ± 0.013^{c}

In the experimental site, flower opening of *Leonurus cardiac* began early in the morning around 6:30 am and had completed by 11 am. The average of flower longevity was 3.52± 0.05, 30; mean ± SE, n. The pollens were available to pollinators 2-3h after the flowers opened. Approximately, 24h after flower opening, the styles were 7.48± 0.01 mm long. The styles continued to elongate and became 8.12± 0.01 mm long after 48 h, a significant growth. Bud burst, shoot proliferation, and leaf expansion began late in February, coinciding with raising the temperature. The first flower buds on the main shoots appeared in 15-20 April and full bloom was observed after 4 weeks (10-15 May). Flowering on lateral branches was started when it terminated in main shoots (10-17 June) and extended until 20-30 August. The date of the peak flowering was 10-15 May, and then the flowers on the main stems were dried, followed by the start of flowering on lateral branches.

Seed set on main shoots occurred on 16-22 July, while on lateral branches on 25-30 September. The plants continued to growth for 20-30 days. After that, the aerial organs dried, and the plant became dormant. The flowering period of this species in Karaj is long (four months).

B. Pollen germination and stigma receptivity

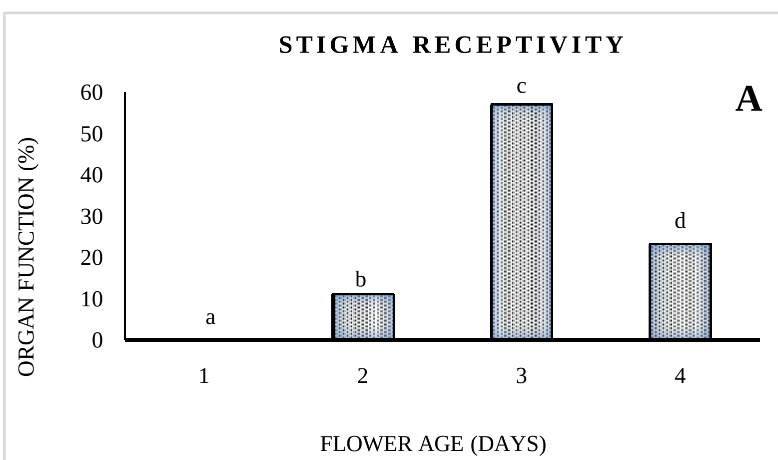
The time of stigma receptivity was measured by seed production percentage. As the flower aged, the stigma receptivity increased significantly ($P < 0.01$; Fig. 2 (A)). The peak of seed production occurred within 48h after anthesis (three-day old flowers) and decreased dramatically in next 12h. With increasing the stigma receptivity, the style became longer, and the stigma's lobes opened.

The stigma receptivity and style length were positively correlated. The figure 1 (A) show in vitro pollen germination of *L. cardiaca.* The highest percentage of in-vitro pollen germination occurred 2 hours after flower opening, synchronizing with anther dehiscence, and then reduced significantly, reaching its minimum after 72 hours (P < 0. 01; Fig. 2 (B)). Pearson correlation coefficient showed that style length had a positive meaningful correlation with stigma receptivity, while it had negative correlations with flower age and pollen germination (Table 3).

Fig. 1. Floral organs, flower and inflorescence of *Leonurus cardiaca*, (A) inflorescence; (B) flower with anther and stigma; (C) in vitro pollen germination.

Table 3: Correlation coefficients among style length, stigma receptivity and pollen germination in *Leonurus cardiac.*

Stigma receptivity	Pollen germination	Style length	
		1	Style length
	1	-0.977 **	Pollen germination
1	-0.655 *	0.779 **	Stigmatic receptivity



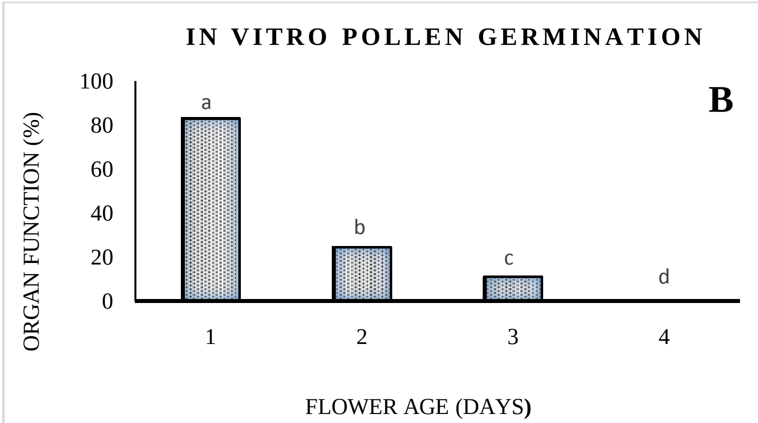


Fig. 2. (A and B). Sexual functioning of flowers *Leonurus cardiaca*. Significant differences in female function and male according with flower age ($P < 0.01$).

C. Pollen-ovule levels

The ratio of the number of pollen grains per flower to the number of ovules per flower (2021.167 ± 77.38) revealed that, based on Cruden classification of reproductive systems of plants, *L. cardiaca* is a facultative xenogamous plant (Table 4).

Table 4: Pollen grain number per flower, number of ovules per flower, number of Anther per flower and pollen-ovule ratio (P/O) in *Leonurus cardiaca.*

Pollen-Ovule ratio (mean ± S.E)	Pollen grain number (mean ± S.E)	Ovule number	Anther number
2021.167 ± 77.38	8084.668 ± 309.52	4	4

DISCUSSION

The structural organization of the flower, including those of the stigma and the style, of *Leonurus cardiaca* is comparable with those of the other members of Lamioideae subfamily (Jamzad, 2012), indicating that these features are conserved. The flowering started in mid-May and ended in late-August. Flower longevity is often short (a few days) in plants (Primack, 1985). The flowers of *Leonurus cardiaca* last 3.5 days, which is comparable with those of other members of Lamiaceae such as *Salvia verbenacea* (Navarro, 1977) and *S. sclareoides* (Jorge *et al*., 2014), whose flowers last for 2.9 ± 0.3 days and 2.94 ± 0.16 days, respectively.

In *L. cardiaca*, the release of pollen and stigma receptivity are asynchronous, and anther dehisces before the stigma becomes receptive. The time gap between male and female phases is about 2-3 days. The flowers pass through sequence of male, bisexual, and female phases.

Breeding system in *Leonurus cardiaca* favours out crossing through the dehiscence of anthers before the stigma is receptive (protandry).

In protandrous species are two sex phases (male and female), which were separated in terms of time. Pollen dispersal starts 2-3 h after flower opening, while the style is situated below the level of anthers, and the stigma branches are closed. At this stage, the flowers are totally male and disperse pollens. At female stage, the style increases in length, and stigma branches separate (after 2 days). It seems that autogamy can take place at the second day after anthesis, when flowers act as bisexual, which means both stigma and pollens are functional. Lloyd and Webb (1986) have suggested that the separation of pollen and stigma acts in general to reduce self-interference and often reduces self-fertilization. To avoid self-pollination, a negative correlation between stigma receptivity and pollen viability exists in protandrus species, although it cannot prevent the pollination of the flower by pollens of the other flowers on the same plant (geitenogamy). Rodriguez-Riano and Dafni (2007) pointed out that in *Origanum syriacum*, self-pollination is avoided by dichogamy (a negative correlation between stigma receptivity and pollen viability), and the style length is significantly positively correlated with stigma receptivity.

Various authors have used the P/O ratio directly as a predictor of the breeding system. Cruden (1977) developed the method of pollen-ovule levels as an estimate of breeding system using 80 different species from a range of genera and provided a table containing the pollen ovule levels for six different breeding systems: cleistogamous, obligate inbreeder, facultative in breeder, outcrosser and obligate outcrosser. He reports that outcrossing plants produce large amounts of pollens to increase the chance of pollen transferring to the stigma. Mione and Anderson (1992) in some species of *Solanum*, Jurgens *et al*. (2002) in five genera of Cariophylloidae, Garcia *et al.* (2014) in genus Passiflora, and Jurgens *et al*. (2014) in Conophytum used pollen-ovule levels as a measure for the estimation of the breeding system. Based on this ratio, *Leonurus cardiaca* is a facultative outcrosser like many other species of Lamiaceae (Claben-Bockhoff, 2007).

REFERENCES

Barrett, S.C.H. (2010). Understanding plant reproductive diversity. *Philos Trans Ser B*. **365**: 99-109.

Bennett, S.J. (2011). Pollen-ovule ratios as a method of estimating breeding systems in Trifoliumpasture species. *Aust J Agr Res.* **50**: 1443-1450.

Claben-Bockhoff, R. (2007). Floral Construction and pollination biology in the Lamiaceae. *Ann Bot-London*.**100**: 359-360.

Cronquist, A. (1981). An integrated system of classification of flowering plants. Columbia University Press, New York. 1262 pages.

Cruden, R.W. (1977). Pollen-Ovule ratios: A conservative indicator of breeding systems in flowering plants. *Evolution.* **31**: 32-46.

Cruden, R.W, Hermanutz, L. and Shuttleworth, J. (1984). The pollination biology and breeding system of *Monarda fistulosa* (Labiatae). Oecologia (Berlin). **64**: 104-110.

Dane, F, Olgun, G. and Dalgç, O. (2004). In vitro pollen germination of some plant species in basic culture medium. *J Cell Mol Biol.* **3**: 71-76.

Garcia, M.T.A, Miguez, M.B. and Gottsberger, G. (2014). Pollen: ovule ratio and its relationship with other reproductive traits in some *Passiflora* species (Passifloraceae). *Ann J Bot Madrid*. **71**: 1-8.

Hedge, I.C. (1992). A global survey of the biogeography of the Labiatae. Pages 7-17 in RM Harley, T Reynolds, eds. Advances in Labiatae science. Royal Botanic Gardens, Kew, London.

Huck, R.B. (1992). Overview of pollination biology in the Lamiaceae. Pages 167-181 in RM Harley, T Reynolds, eds. *Advances in Labiatae Science. Royal Botanic Gardens*, Kew, London.

Hidalgo, J.P. and Ubera, J.L. (2001). Inbreeding depression in *Rosmarinus officinalis* L. *Int J Dev Biol.* **45**: 43-44.

IBM Corp. (2013). IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Jamzad, Z. (2012). Lamiaceae in Assadi M, Maassoumi A, Mozaffarian V. (eds.), Flora of Iran, no. 76, Research Institute of Forest and Rangelands, Tehran, Iran.

Jorge, A, Loureiro, J. and Castro, S. (2014). Flower biology and breeding system of *Salvia sclareoides* Brot. (Lamiaceae). *Pl Syst Evol* DOI 10.1007/s00606-014-1169-7.

Jurgens, A, Witt, T. and Gottsberger, G. (2002). Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: correlation with breeding system, pollination, life form, style number, and sexual system. *Sex Plant Reprod*. **14**: 279-289.

Jurgens, A. and Witt, T. (2014). Pollen-ovule ratios and flower visitors of day-flowering and night-flowering Conophytum (Aizoaceae) species in South Africa. *J Arid Environ.* **109**: 44-53.

Lloyd, D.G. and Webb, C.J. (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. *New Zeal J Bot.* **24**: 135-162.

McIntosh, M.E. (2002). Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). *Plant Ecol.*, **159**: 1-13.

Mione, T. and Anderson, G.J. (1992). Pollen-Ovule ratios and breeding system evolution in Solanum Section Basarthrum (Solanaceae). *Am J Bot.* **79**: 279-287.

Milkowska-Leyck, K, Barbara Filipeka, B. and Strzelecka, H. (2002). Pharmacological effects of lavandulifolioside from *Leonurus cardiaca*. *J Ethnopharmacol*. **80**: 85-90.

Navarro, L. (1997). Is the dichogamy of *Salvia verbenaca* (Lamiaceae) an effective barrier to self-fertilization? *Pl Syst Evol.* **207**: 111-117.

Primack, R.B. (1985). Longevity of individual flowers. *Annu Rev Ecol Syst*. **16**: 15-37.

Rathcke B, Lacey EP, (1985). Phenological patterns of terrestrial plants. *Annu Rev Ecol Syst*. **16**: 179-214.

Rodriguez-Riano, T. and Dafni, A. (2007). Pollen - Stigma interference in two gynodioecious species of Lamiaceae with intermediate individuals. *Ann Bot-London*. **100**: 423-431.

Russian Pharmacopoeia. (1968). Gosudarstwiennaja Farmak opea SSSR (X), Medgiz, Moskwa. 347-348.

Solomon Raju, A.J. and Subba Reddi. (1989). Pollination biology of *Anisomeles indica* and *A. molabaria* (Lamiaceae). *Plant spec boil*. **4**: 156-167.

Subashi, U. and Guvensen, A. (2011). Breeding systems and reproductive success on *Salvia smyrnaea*. *Turkish J Bot*. **35**: 681-687.

Tandon, R, Shivanna, K.R, and Mohan Ram, H.Y. (2003). Reproductive biology of *Butea monosperma* (Fabaceae). *Ann Bot-London*. **92**: 715-722.

Wojtyniak, K, Szymanski, M. and Matlawska, I. (2013). *Leonurus cardiaca* L. (Motherwort): a review of its phytochemistry and pharmacology. *Phytother Res.* **27**: 1115-1120.

Yeom, F.K.S, Lau, C.Y, Tawan, C.S, Pawazan, A.M. and Sim, S.L. (2006). Sustainable management and utilization of medicinal plant resources. *Proceeding of the international conference on medicinal plants*. p. 158-169.