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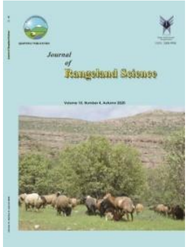

Research and Full Length Article:

Flowering Features and Breeding Systems of Seven Native *Salvia* Species in Iran

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Abstract. Before starting any classical breeding program on plants, it is necessary to dominate the fertilization behavior of that plant. Despite a large number of *Salvia* species in the flora of Iran, there is little information about the breeding system of this genus. In the current study, the flower specifications and breeding system of seven *Salvia* species (Lamiaceae) have been described during 2017-2018. Plants phenology and some flower features were recorded as well as hand pollination used for fertility investigation. The results showed that all species had long flowering periods. The highest flowering period (41 days) was observed in *S. virgata*. The most frequent pollinators were bees and flies. *Salvia virgata* and *S. nemorosa* showed about 90% survival to the new habitat. Two species including *S. atropatana* and *S. syriaca* had heterostylous flowers (long-styled morph). The results of manual pollination indicated that open pollination provided the best treatment with the highest average of seed set (73.63%) in all species. The seed set in the xenogamy treatment of different species and accessions was in the range of 10.2 (*S. syriaca*) to 32.5% (*S. frigida*). It was observed that seed set among different species in geitonogamy and spontaneous autogamy varied widely from 2.60 - 17.30% and 2.98 - 12.18%, respectively. It was concluded that *Salvia* species in the present study were relatively self-incompatible (ISI) and out-cross. They need pollinators to adequate fertility. They did not observe any correlations between ISI and heteromorphy.

Key words: Flower biology, Flower morphology, Hand pollination, Heterostyly, Self-compatible

Introduction

The genus *Salvia* L. (Lamiaceae) is one of the most interesting plant genera with a variety of annual, biennial, perennial, and shrub forms. It has been shown that there are almost 1000 species of *Salvia* worldwide (Claßen-Bockhoff *et al.,* 2003). According to recent reports, there are 61 species of *Salvia* genus in Iran, among which 18 species are endemic (Jamzad, 2013). The use of *Salvia* species dates back to ancient times, and they are now being used for different purposes such as traditional medicines, cosmetic, and food industries (Bahadori *et al.,* 2017) as well as ornamental plants (Karabacak *et al.,* 2009).

In flowering plants, there is a wide variety of reproductive systems and pollinators, ranging from optional self-pollination to obligate cross-pollination (Qu and Widrlechner, 2011). This variation is affected by several factors including floral structure (form and functional characteristics of sexual units), environmental conditions, presence or absence of pollinator, etc. (Castro *et al.*, 2008a). It seems that besides the floral structure concerning time (synchrony in gender function) and place (flower morphology and inflorescence structure), the presence of visitors can also affect the mating system type (Dudash and Fenster, 2001; Castro *et al.,* 2008b). Understanding the flowering biology, reproductive systems, and pollination mechanism is essential to the management and protection of the ecosystem and selection of the best mating programs in breeding programs (Evans *et al.,* 2003; Nautiyal *et al.,* 2009; Nebot *et al.*, 2016).

The breeding of medicinal plants has attracted the growing attention of researchers in the past decade. Compared to crop plants, the breeding programs of medicinal plants are still in nascent stages due to high biological and biochemical variations (Jorge *et al.*, 2015). Since breeding programs, especially in medicinal plants are expensive, suitable mating can help reduce the costs of breeding programs.

Different mechanisms such as cleistogamy as a method of self-pollination without flower opening (Koike *et al.,* 2015); autogamy as the fertilization of a flower by pollen from the same flower (Galen *et al.*, 2017); geitonogamy as the fertilization of a flower by pollen from another flower and the same plant (De Jong *et al.*, 1993); xenogamy as the fertilization of a flower by the pollen of a flower from a genetically different plant (Cruden and Lyon, 2019) and apomixis, asexual reproduction through seed (Singh *et al.*, 2007); they affect the seed production of *Salvia* species (Barrett *et al.,* 2000; Jorge *et al.*, 2015). However, it has been shown that *Salvia* species are mostly self-compatible (Song *et al.,* 2009). In most cases, diverse phenomena such as dichogamy (male and female organs are frequently separated in time), hercogamy (male and female organs are frequently separated in space), gynodioecy (in which individuals within a single population produce either female or bisexual flowers) and self-incompatibility promote outcrossing in *Salvia* genus (Navarro, 1997; Takano, 2013).

Many *Salvia* species are characterized by attractive flowers, high nectar, and long blooming season (Da Silva *et al.,* 2005). Thus, the most frequent visitors of this genus are bees (Hymenoptera) and flies (Diptera); though not all of them take part in pollination (Celep *et al.*, 2014). The role of other pollinators such as birds in the pollination of this genus has also been reported (Sanchez *et al.,* 2002).

So far, many studies have explored different subjects of floral biology in *Salvia* genus including pollination ecology: *Salvia verbenaca* L. (Navarro, 1997), *Salvia brandegeei* Munz (Barrett *et al.,* 2000), *Salvia splendens* Sellow ex Roem. & Schult. (Miyajima, 2001), *Salvia nipponica* Miq. (Miyake and Sakai, 2005), *Salvia haenkei* Benth. (Wester and Claßen-Bockhoff, 2006b) and pollen transfer mechanisms: a review of *Salvia* species (Wester and Claßen-Bockhoff, 2007); bee-and bird-pollinated species of *Salvia* (Wester and Claßen-Bockhoff, 2006a); seed production: *Salvia splendens* (Sanchez *et al.*, 2002) and breeding system*: S. smyrnaea* Boiss. (Subaşi and Güvensen, 2011).

Even though the high diversity of *Salvia* genus in Iran and most of them have high potential to be used as medicinal and/or ornamental plants, but there is a paucity of studies on reproductive biology and pollination of *Salvia* species. In this research, we collected seeds of seven *Salvia* species from their natural habitats. As far as the researchers are concerned, few studies have been undertaken on the reproductive systems and domestication of these species so far. As a result, we investigated the flowering biology and reproductive systems of the species mentioned above in un-natural habitat (cultivated conditions). The present research seeks to obtain the following goals in seven native *Salvia* species: 1) Study seedling establishment and flowering features as a first step for domestication, 2) Looking for heteromorphy flowers as a reason for outcrossing, 3) a description of the reproductive system to determine the role of pollination in fruit and seed production using hand pollinations. 4) Take a conclusion about the self-compatibility rate in various species as a valuable trait in hybridization. With this information available, it is possible to select better crossing methods.

Materials and Methods

Plant materials and study area

The study was carried out in the Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran, from December 2017 to October 2018. The study area was at an elevation of about 1039 m with coordinates of 36º17´25´´ N and 59º35´45´´ E. In the first step, seeds of seven *Salvia* species including *S. virgata* (with four accessions), *S. frigida*, *S. nemorosa*, *S. atropatana*, *S. macrosiphon*, *S. sclarea*, and *S. syriaca* were collected from different geographical regions. All species were identified at Ferdowsi University of Mashhad Herbarium (FUMH). Further information about the origins and accessions of these species is given in Table 1.

Table 1. **The collected sites and some features of *Salvia* species of Iran.**

Species	Collected location (Province- City)	Longitude	Latitude	Altitude m
S. virgata (A_1)	Khorasan-e- Shomali-Darkesh	37º26´31´´	56º45´08´´	1239
S. virgata (A_2)	Fars-Eghelid	30º30´45´´	52º43´51´´	2559
S. virgata (A_3)	Isfahan-Fereydan	33º08´45´´	50º16´22´´	2360
S. virgata (A_4)	Isfahan-Afous	33º00´14´´	50º00´42´´	2557
S. frigida	Azarbayijan-Targara	37º12´54´´	44º51´33´´	1995
S. nemorosa	Isfahan-Buin-e-Meyandasht	33º01´00´´	50º20´16´´	2330
S. atropatana	Khorasan-e-Shomali-Lojali	37º43´18´´	57º54´42´´	1723
S. macrosiphon	Khorasan-e-Razavi- Kalatenaderi	36º36´35´´	59º54´54´´	1821
S. sclarea	Khorasan-e- Shomali-Reein	37º23´11´´	57º23´07´´	1889
S. syriaca	Isfahan-Buin-e-Meyandasht	33º01´00´´	50º20´16´´	2699

Seed germination and seedling production

In the second step, seeds of different *Salvia* species were treated by various methods such as washing, cold treatment (stratification), scarification (sand and sulfuric acid), Gibberellic acid (GA_3) and KNO_3. The treated seeds were cultivated in Petri dishes and placed in a germinator with the photoperiod of 16/8 h and a temperature of 25 ºC. Seed germination was recorded for 20 days.

In the third step, seedling survival was investigated in different media. Seedlings with four true leaves were transferred to pots containing soil made of diverse ratios of clay, silt and sand, coco-peat, perlite and peat moss in the greenhouse. After growing as high as 15 cm or reaching the 10-leaf stage, 16 seedlings of each genotype were planted in plots (1.5 × 1.5m) with three replications (Fig. 2-A & B) - and other common cultivating practices were performed. Some morphological features, survival rate, and breeding system were studied in the growing season.

Plant phenology and flower morphology

The life cycle of species was pursued in the second year. Three main steps including rosette, flowering date, and seed ripening were considered. Ten flower buds (each on a different plant) were tagged and monitored daily throughout the flowering period to check the opening and abscission days. Furthermore, morphometric analyses were performed for ten flowers belonging to each population and species. Some traits such as the length of the pistil and stamen also distance between anthers tip and stigmas surface were measured by a binocular microscope for heteromorphic rate estimation. The flower visitors were monitored three times a day at 9 am, 2 pm and 6 pm for two weeks.

Breeding system

Pollination treatments were designed to investigate the breeding system of *Salvia* species based on the studies of Dafni (1992) and Gan *et al.* (2013), which were undertaken from April 20 to September 10, 2016. Flowers were bagged by a mosquito net to prevent insect visiting before anthesis. For manual pollination, bags were opened and the following treatments were applied: 1) Spontaneous autogamy, flowers were bagged without emasculation;

2) Apomixes, flowers were bagged with a fine nylon mesh after emasculation to determine whether there was any apomixis or not;

3) Geitonogamy, flowers were first emasculated and then out-crossed by pollens collected from flowers of the same plant by manual pollination, and finally re-bagged;

4) Xenogamy, flowers were first emasculated, then out-crossed with a fresh pollen mixture collected from other plants using manual pollinations, and finally re-bagged;

5) Open pollination (control), no procedures were applied to flowers and they were exposed to pollinator visits (natural levels of the fruit set).

In all treatments, flowers were monitored until the seeds were mature. Before nutlet dispersal time, flowers were collected to calculate the number of nutlets developed in each calyx. Each ovule can produce one seed with a maximum seed set of four per flower. Moreover, in order to determine the self-incompatibility index (ISI), the number of fruit set through geitonogamy was divided by the number of fruit set through xenogamy. In this index,

ISI > 1 represents high self-compatibility,

0.2 < ISI < 1 shows the relative self-incompatibility,

ISI < 0.2 indicates severe self-incompatibility, and

0 implies complete self-incompatibility (Zapata and Arroyo, 1978).

Statistical analysis

Mean and standard deviations were calculated in Microsoft Excel 2013. The fruit set of different *Salvia* species in manual pollination treatments was analyzed by one-way ANOVA, followed by LSD test using JMP. 8 statistical software at a significant level of α= 0.01.

Results and Discussion

Seed germination and seedling growth

Germination study exhibited that washing seeds for 24 h and applying 100 ppm GA_3 exerted the highest effect on seed germination (data not shown). *Salvia* species studied in this paper had a dormancy period, which can be removed by seed soaking in GA_3. Plants develop various dormancy systems for their survival. It is clear that seeds collected from the natural habitat contain germination inhibitor compounds, seed washing allows eliminating some inhibitor substances that reside in the seed coat (Bewley and Black, 1994; Angeline and Ouma, 2008). The results suggested that GA_3 improved seed germination, probably due to the satisfaction of chilling requirements inherent to this kind of seeds. In the meantime, environmental conditions and hormonal balance of seeds also play a critical role in seed germination (Asghari *et al.*, 2015; Shu *et al.*, 2016).

Investigation of seedling survival revealed that seedlings in early growth stage required medium to high porosity (high permeability to water and air) like natural habitat, but they were sensitive to dry and high EC at these stages. In this study, the survival of different *Salvia* species on the farm was investigated for 18 months (Table 2). The results suggested that the highest survival rate belonged to *S. virgata* (A_2) (91.6%) followed by *S. nemorosa* (83.3%), and the lowest survival rate was observed in *S. atropatana* (16.6%). The survival rate of different species depends on the survival rate of plants and production of live seeds.

Table 2. Plant futures and survival of different *Salvia* species at Mashhad weather condition.

Species	Plant Height (cm)[a]	Flower color	100 Seed weight (g)	Survival rate %
S. virgata (A_1)	47.3 ± 3.9	Violet	0.116	65.0 ± 5.7
S. virgata (A_2)	40.3 ± 2.5	Violet	0.092	91.0 ± 2.4
S. virgata (A_3)	75.7 ± 1.7	Violet	0.095	65.3 ± 2.5
S. virgata (A_4)	65.3 ± 2.5	Violet	0.106	74.7 ± 2.1
S. frigida	44.7 ± 2.1	Violet	0.247	32.3 ± 2.1
S. nemorosa	61.7 ± 2.9	Blue	0.078	83.3 ± 3.9
S. atropatana	37.7 ± 4.9	White	0.415	16.3 ± 2.9
S. macrosiphon	80.3 ± 2.9	White	0.273	33.3 ± 1.2
S. sclarea	130.3 ± 4.5	Violet	0.220	75.3 ± 2.5
S. syriaca	24.3 ± 1.7	White	0.206	25.0 ± 4.1

* Values are means ± SD of three replicates.

Plant phenology and flower morphology

The phenology of Salvia species is shown in Fig. 1. All seedlings were transplanted to the farm on 10 April 2016. *Salvia virgata* (all accessions) and *S. nemorosa* went to flowering stage three months after cultivation and other species flowered in the second year. According to Fig. 1, all species required a special number of leaves and duration before flowering (48 to 96 days). A marked variation in flower duration of different *Salvia* species was observed. The flowering period of *S. atropatana* and *S. virgata* (A1) had the minimum (15 days) and maximum (41 days) longevity (Table 3). The flower longevity plays a crucial role in the number of pollinators visiting a flower as well as the seed set (Castro, 2008a). Increased flowering period led to the enhancement of flower over-lapping and the sharing of the pollen among different plants or species (Claßen-Bockhoff *et al.*, 2004). Furthermore, the flowering period is one of the critical factors in ornamental plants.

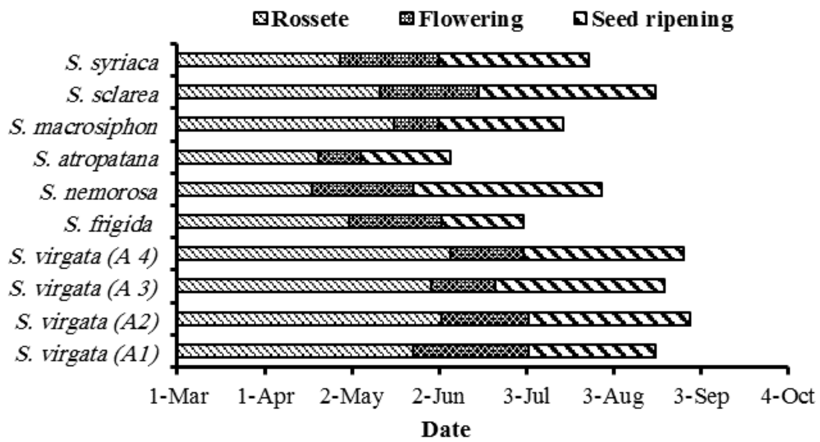

Fig. 1. Comparison of three important phenology steps of *Salvia* species at the second year life cycle. All species transplanted at late April 2016. First March 2017 as start new growth after winter.

The mean distance between anthers tip and stigma surface was 0.0 to 7.1±0.33 mm (Table 3). With the exception of two species, the distance between anther and stigma in other species was due to the reason that stamens mature first (protandry) and then, they release pollen grains before the gynoecium reaches its maximum maturation and elongation. As such, in some studies, the flowering of *Salvia* was divided into various steps including flower opening, male, hermaphrodite, and female stage (Ott *et al.*, 2016). The anther and stigma distance is created after the opening of the flower, so it is not explanatory of heterostyly; although heterostyly was observed in *S. atropatana* and *S. syriaca* (7.1 and 5.2 mm distance between anther and stigma respectively).

Table 3. Flower duration and hetrostylos level of different *Salvia* species at Mashhad weather condition.

Species	Pistil length (mm)	Stamen length (mm)	Distance between Anther and stigma (mm)	Date of start Flowering	Date of end Flowering	Flowering Duration (day)*
S. virgata (A_1)	16.1±0.2	11.1 ± 0.2	2.5 ± 0.08	30-April-17	10-June-17	40.7 ± 2.5
S. virgata (A_2)	18.0±0.5	11.1 ± 0.2	2.4 ± 0.12	16-May-17	16-June-17	30.7 ± 2.9
S. virgata (A_3)	17.9±0.3	12.0 ± 0.3	2.1 ± 0.21	8-May-17	31-May-17	22.7 ± 2.5
S. virgata (A_4)	17.2±0.3	12.0 ± 0.2	2.1 ± 0.08	1- May-17	5-June-17	26.0 ± 2.2
S. frigida	16.0±0.2	10.0 ± 0.2	2.0 ± 0.26	8-May-17	10-June-17	32.7 ± 2.6
S. nemorosa	24.9±0.5	9.1 ± 0.2	1.1 ± 0.08	22-April-17	3- May-17	35.0 ± 2.4
S. atropatana	32.1±0.6	13.1 ± 0.3	7.1 ± 0.33	20-April-17	5-May-17	15.3 ± 2.5
S.macrosiphon	28.1±0.2	11.0 ± 0.3	0.5 ± 0.05	20-May-17	5-June-17	15.7 ± 0.5
S. sclarea	25.0±0.2	10.9 ± 0.1	0.0 ± 0.0	14-May-17	18-June-17	34.7 ± 2.9
S. syriaca	20.9±0.5	9.0 ± 0.3	5.2 ± 0.37	26-April-17	31-May-17	35.3 ± 1.2

*Values are means ± SD of three replicates.

All species had multiple inflorescences per plant, which contained several verticillasters, each with six or more flowers, which in turn contained two-branch stigma and four ovules. Calyx and corolla surface covered with different types of trichomes (Fig. 2- D & E). *Salvia frigida* and *S. atropatana* with three and four months had the shortest life cycle and *S. virgata* and *S. sclarea* with six months had the longest life cycle in the second years. In all mature flowers, pistils were considerably protruded from the corolla tube and stamens contained a large number of pollens (Fig. 2-F). In general, most of *Salvia* flowers are described by movable anthers whose lower lever arms have to be pushed back by the pollinator (see the review of Claßen-Bockhoff *et al.*, 2003). This mechanism was observed in this study (Fig. 2-H & I).

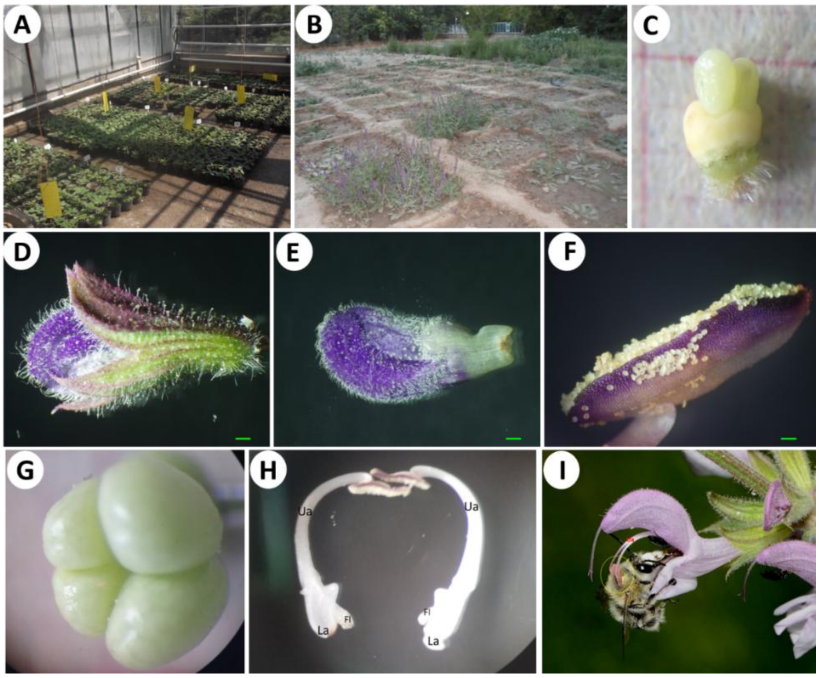

Fig. 2. (A) Salvias seedling production in the greenhouse, (B) *Salvia* seedling transplanting in the farm, (C) Seed formation on only two ovules (spontaneous autogamy treatment), (D) Calyx covered with different trichomes, (E) Corolla covered with palate glandular trichomes, (F) Pollens release after anther maturation (100 ×, S. *nemorosa*), (G) Seed formation on four ovules (open pollination treatment), (H & I) Stamen Lever Mechanism description: Stamens have a small filament (Fl) that fixed in the corolla and two mobile arms, when a pollinator try to collect nectar, push the lower lever arm (La) led to the move down the upper lever arm (Ua) and touch with pollinator body (I).

The results of pollinator observation showed that bees and flies played a key role in pollen transport and seed set. Other insects such as butterflies and beetles were also observed, but it seemed that their bodies had not touched stigma and anther and they served the role of pollen collection or hunting of other insects. Senol *et al.* (2017) reported different insect taxa (special Hymenoptera and Diptera) with various functions in *S. sclarea*. Honeybees were introduced as the most frequency pollinators of *S. apiana* (Ott *et al.,* 2016). According to the previous reports, *Salvia* flowers attract different pollinators that indicate species origin (Claßen-Bockhoff *et al.,* 2004; Senol *et al.,* 2017).

Breeding System

The results of various pollination treatments are presented in Tables 4, 5, and 6. There is a significant difference between pollination treatments (apomixis can be excluded) and species (p<0.01). As it can be seen, the highest fruit set obtained in the open pollination treatment ranged from 51.5 to 90.8% (Table 4). Open pollination leads to frequent flower visit by pollinators and finally, effective pollination and seed set. A comparison of different species exhibited that three genotypes containing *S. virgata* (accession number 1 and 3) and *S. macrosiphon* had a seed set of more than 80% in the open pollination treatment. About 98% of successful pollination has been reported for *S. sclarea* in open pollination treatment (Senol *et al.*, 2017).

Table 4. The results of open pollination and spontaneous autogamy treatments in *Salvia* species.

Species	Open pollination				Spontaneous autogamy			
	Treated Flowers No	Max. seeds Number	Full Seeds %	Aborted Seeds %	Treated Flowers No	Max. seeds Number	Full Seeds %	Aborted Seeds %
S. virgata (A_1)	38	152	90.3[a]	5.9[d]	33	132	3.0[d]	1.5[g]
S. virgata (A_2)	52	208	66.6[d]	6.8[d]	35	140	12.2[a]	2.8[f]
S. virgata (A_3)	37	148	81.1[b]	9.4[bc]	70	280	6.8[c]	1.0[g]
S. virgata (A_4)	20	80	66.5[d]	8.7[c]	57	228	12.1[a]	13.3[a]
S. frigida	37	148	75.7[c]	11.5[b]	55	220	9.1[b]	3.2[ef]
S. nemorosa	44	176	67.2[d]	17.0[a]	42	168	6.8[c]	4.6[d]
S. atropatana	28	112	76.9[c]	8.6[c]	32	128	4.3[d]	4.0[de]
S. macrosiphon	32	128	88.5[a]	5.9[c]	35	140	8.3[b]	10.7[b]
S. sclarea	42	168	75.5[c]	7.2[cd]	30	120	11.7[a]	7.5[c]
S. syriaca	55	220	51.5[e]	14.6[a]	42	168	3.4[d]	2.4[f]

* Different letters in each column show significant differences at P < 0.01.

A comparison of other treatments showed that the highest seed sets were observed in xenogamy, geitonogamy, and spontaneous autogamy, respectively (Tables 5 & 6). Seed reproduction by spontaneous autogamy was extremely low between 2.98 - 12.2%, underscoring the importance of pollination. It may be due to the heterostyly of flowers (pin) or dichogamy (male and female organs are frequently separated in time), which increased the efficiency of cross-pollination (Franklin-Tong, 2008). A comparison of geitonogamy and xenogamy showed that pollen grains from other sources (other plants of the same species) had priority in seed set driven by self-incompatibility. Therefore, the origin of pollen grains can significantly affect seed production. Lack of fruit set was observed in the apomixis treatment except for accession No 4 (A_4) of *S. virgata* (2.89%); however, further studies are required to confirm this observation (Table 6).

Table 5. The results of geitonogamy and xenogamy treatments in *Salvia* species

Species	Geitonogamy				Xenogamy			
	Treated Flowers No	Max. seeds Number	Full Seeds %	Aborted Seeds %	Treated Flowers No	Max. seeds Number	Full Seeds %	Aborted Seeds %
S. virgata (A_1)	22	88	17.3[a]	2.2[f]	22	88	26.8[b]	3.3[c]
S. virgata (A_2)	15	60	17.2[a]	5.0[c]	15	60	17.7[de]	3.8[b]
S. virgata (A_3)	31	124	2.6[f]	3.3[d]	26	104	24.0[c]	0.0[g]
S. virgata (A_4)	24	96	15.2[b]	0.0[g]	24	96	23.3[c]	1.0[f]
S. frigida	15	60	3.2[f]	6.0[b]	15	60	32.5[a]	0.0[g]
S. nemorosa	25	100	2.6[f]	2.0[f]	25	100	14.5[fg]	1.5[e]
S. atropatana	36	144	13.5[c]	2.8[e]	20	80	15.3[ef]	2.7[d]
S. macrosiphon	40	160	11.2[d]	0.0[g]	28	112	18.2[d]	5.5[a]
S. sclarea	38	152	8.6[e]	0.0[g]	22	88	12.2[gh]	0.0[g]
S. syriaca	35	140	2.8[f]	7.6[a]	18	72	10.2[h]	2.5[d]

*Different letters in each column show significant differences at $P < 0.01$.

Although the production of four seeds per flower was observed only in open pollination (Fig. 2- C & G), the average seed production was below the theoretical maximum. Thus, besides the pollination factor, other parameters such as plant genetics and environmental conditions are involved in plant reproduction. We did not observe any correlation between seed set and flowering period, but this clear long flowering period increases the possibility of visit flowers by pollinators.

At the end of pollination experiments, ISI was calculated (Table 6). The results indicated that ISI varied in different *Salvia* species. *Salvia virgata* (A_3), *S. frigida* and *S. nemorosa* demonstrated intense self-incompatibility. Most species, nonetheless, were relatively self-incompatible. In previous studies, a variety of self-compatibility or incompatibility has been reported. For example, Subaşi and Güvensen (2011) reported partial self-incompatibility in *Salvia smyrnaea* and partial self-compatibility in *Eriope blanchetii* Benth. (Lamiaceae) (Da Silva *et al.*, 2005). Şenol *et al.* (2017) also reported that *S. sclarea* was partly self-incompatible (ISI = 0.49). In *Salvia splendens*, the rate of seed production in bagged plants and open-pollinated was 0.76 and 1.30 seeds per flower, respectively (Miyajima, 1996). Sanchez *et al.* (2002) contended that the origin of pollen grains did not influence seed set in *S. splendens*. Navarro (1997) argued that *Salvia verbenaca*, due to its self-compatibility, did not require an insect for pollination. Also, the study on the breeding system and pollinators of *S. apiana* indicated that it was self-compatible and plants suffered from the pollinator limitation (Ott *et al.*, 2016).

Table 6. Seed formation in apomixis treatment and the rate of self- incompatibility in *Salvia* species

Species	Apomixis				Xenogamy	
	Treated Flowers No	Max. Seeds Number	Full Seeds %	Aborted Seeds %	ISI	Intensity of self-incompatibility
S. virgata (A_1)	22	88	0	0	0.65	Relatively self-incompatible
S. virgata (A_2)	16	64	0	0	0.99	Relatively self-incompatible
S. virgata (A_3)	26	104	0	0	0.11	Severely self-incompatible
S. virgata (A_4)	26	104	2.89	0	0.65	Relatively self-incompatible
S. frigida	31	124	0	0	0.10	Severely self-incompatible
S. nemorosa	27	108	0	0	0.18	Severely self-incompatible
S. atropatana	24	100	0	0	0.91	Relatively self-incompatible
S. macrosiphon	22	88	0	0	0.61	Relatively self-incompatible
S. sclarea	30	120	0	0	0.71	Relatively self-incompatible
S. syriaca	18	72	0	0	0.28	Relatively self-incompatible

*Different letters in each column show significant differences at $P < 0.01$; ISI, Index of self-incompatible

In the past three decades, few reports have been published on heterostyly in Lamiaceae family, mainly because it was assumed that heterostyly rarely occurred in families with zygomorphic flowers. Barrett *et al.* (2000) heterostyly in *Salvia brandegeei* Munz reported that it was caused by the environmental condition. In the present study, long-styled morphs were observed for *S. atropatana* and *S. syriaca*. Thus, one reason for cross-pollination of some *Salvia* species is their heterostylous flowers. Since the heterostyly of flowers hinders the transfer of pollen to stigma surface, the role of pollinators is crucial and lack of pollinators may lead to low seed set. Thus, seed production can be improved by self-compatibility. Sanchez *et al.* (2002) also reported that spatial separation of anthers and stigmas prevented the spontaneous selfing in *Salvia* species.

There were also variations in the seed set of different accessions (Table 4). Among different accessions of *S. virgata*, the highest seed set was observed in A_1 by open pollination (90.30%), suggesting that geographical regions can influence the mating system by affecting the evolution of the flower structure. The remarkable point was the diversity of ISI in the same species (*S. virgata* and *S. frigida*), which highlights the critical role of pollen source in the reproduction of flowers. It also may be due to the experimental error.

Fruit set was higher in treatments with diverse pollen sources, indicating that pollen limitation caused low seed set, and pollinator limitation played an important role in this regard. The flower is the structure that is directly involved in pollinator attraction, and its morphological and functional traits affect the reproduction of the plant. Reduced seed production in bagged flowers could be attributed to the lack of effective pollination. Therefore, external pollinating agents are essential for the optimal seed set. The results can help conduct more in-depth studies on the breeding system and provide strategies for effective genetic conservation and crop improvement. Indeed, it is necessary to study floral dimorphism and sexual polymorphism in these species.

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Reference

Angeline, O., Ouma, G., 2008. Effect of washing and media on the germination of papaya seeds. Journal of Agricultural and Biological Science, 3(1): 8–11.

Asgari, M., Nasiri, M., Jafari, A.A., Flah Hosseini L., 2015. Investigation of chilling effects on characteristics of seed germination, vigor and seedling growth of *Nepeta* spp. species. Journal of Rangeland Science, 5(4): 313-324.

Bahadori, M.B., Dinparast, L., Zengin, G., Sarikurkcu, C., Bahadori, S., Asghari, B., Movahhedin, N., 2017. Functional components, antidiabetic, anti-alzheimer's disease, and antioxidant activities of *Salvia syria*ca L. International Journal of Food Properties, 20(8): 1761–1772.

Barrett, S.C.H., Wilken, D.H., Cole, W.W., 2000. Heterostyly in the Lamiaceae: The case of *Salvia brandegeei*. Plant Systematics and Evolution, 223: 211–219.

Bewley, J.D., Black, M., 1994. Seeds, Physiology of Development and Germination. Springer, Boston, MA.

Castro, S., Silveira, P., Navarro, L., 2008a. How flower biology and breeding system affect the reproductive success of the narrow endemic *Polygala vayredae* Costa (Polygalaceae). Botanical Journal of the Linnean Society, 157(1): 67–81.

Castro, S., Silveira, P., Navarro, L., 2008b. Effect of pollination on floral longevity and costs of delaying fertilization in the out-crossing *Polygala vayredae* Costa (Polygalaceae). Annual of Botany, 102(6): 1043–1048.

Celep, F., Atalay, Z., Dikmen, F., Doğan, M., Classen-Bockhoff, R., 2014. Flies as pollinators of melittophilous *Salvia* species (Lamiaceae). American Journal of Botany, 101(12): 2148–2159.

Claßen-Bockhoff, R., Wester, P., Tweraser, E., 2003. The staminal lever mechanism in *Salvia* L. (Lamiaceae) - A review. Plant Biology, 5: 33–41.

Claßen-Bockhoff, R., Speck, T., Tweraser, E., Wester, P., Thimm, S., Reith, M., 2004. The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? Organisms Diversity & Evolution, 4(3): 189–205.

Cruden, R.W, Lyon, D.L., 2019. Facultative Xenogamy: Examination of A Mixed Mating System. In The Evolutionary Ecology of Plants (pp. 171-207). CRC Press.

Dafni, A., 1992. Pollination Ecology: A Practical Approach. IRL Press at Oxford University Press, UK, 250 pp.

Da Silva, F.O., Viana, B.F., Jacobi, C.M., 2005. Floral biology of *Eriope blanchetii* (Lamiaceae) in coastal sand dunes of NE Brazil. Austral Ecology, 30(3): 243–249.

De Jong, T.J., Waser, N.M., Klinkhamer, P.G., 1993. Geitonogamy: the neglected side of selfing. Trends in Ecology & Evolution, 8(9): 321-325.

Dudash, M.R., Fenster, C.B., 2001. The role of breeding system and inbreeding depression in the maintenance of an outcrossing mating strategy in *Silene virginica* (Caryophyllaceae). American Journal of Botany, 88(11): 1953–1959.

Evans, M.E.K., Menges, E.S., Gordon, D.R., 2003. Reproductive biology of three sympatric endangered plants endemic to Florida scrub. Biological Conservation, 111(2): 235–246.

Franklin-Tong, E., 2008. Self-Incompatibility in Flowering Plants: Evolution, Diversity, and Mechanisms. Springer, Birmingham.

Galen, C., Storks, L., Carpenter, E., Dearborn, J., Guyton, J., O'Daniels, S., 2017. Pollination mechanisms and plant-pollinator relationships. Master Pollinator Steward Program. Published by University of Missouri Extension, M402.

Gan, X., Cao, L., Zhang, X., Li, H., 2013. Floral biology, breeding system and pollination ecology of an endangered tree *Tetracentron sinense* Oliv. (Trochodendraceae). Botanical Studies, 54(1): 50.

Jamzad, Z., 2013. A survey of Lamiaceae in the flora of Iran. Rostaniha, 14(1): 59–67

Jorge, A., Loureiro, J., Castro, S., 2015. Flower biology and breeding system of *Salvia sclareoides* Brot. (Lamiaceae). Plant Systematics and Evolution, 301(5): 1485–1497.

Karabacak, E., Uysal, I., Doğan, M., 2009. Cultivated *Salvia* species in Turkey. Biological Diversity and Conservation, 2: 71-77.

Koike, S., Yamaguchi, T., Ohmori, S., Hayashi, T., Yatou, O., Yoshida, H., 2015. Cleistogamy decreases the effect of high temperature stress at flowering in rice. Plant Production Science, 18(2):111-117.

Miyajima, D., 1996. Seed-producing system in Salvias. Journal of the American Society for Horticultural Science, 121(3): 419–422.

Miyajima, D., 2001. Floral variation and its effect on self-pollination in *Salvia splendens*. Journal of Horticultural Science and Biotechnology, 76(2): 187–194.

Miyake, Y.C., Sakai, S., 2005. Effects of number of flowers per raceme and number of racemes per plant on Bumblebee visits and female reproductive success in *Salvia nipponica* (Labiatae). Ecological Research, 20(4): 395–403.

Nautiyal, B.P., Nautiyal, M.C., Khanduri, V.P., Rawat, N., 2009. Floral biology of *Aconitum* *heterophyllum* wall.: A critically endangered alpine medicinal plant of Himalaya, India. Turkish Journal of Botany, 33: 13–20.

Navarro, L., 1997. Is the dichogamy of *Salvia verbenaca* (Lamiaceae) an effective barrier to self-fertilization? Plant Systematics and Evolution, 207: 111–117.

Nebot, A., Cogoni, D., Fenu, G., Bacchetta, G., 2016. Floral biology and breeding system of the narrow endemic *Dianthus morisianus* Vals. (Caryophyllaceae). Flora, 219: 1–7.

Ott, D., Hühn, P., Claßen-Bockhoff, R., 2016. Salvia apiana, A carpenter bee flower? Flora, 221: 82–91.

Qu, L., Widrlechner, M.P., 2011. Variation in the breeding system of *prunella variation*. Hortscience, 46(5): 688–692.

Sanchez, L.A., Picado, A., Sommeijer, M.J., Slaa, E.J., 2002. Floral biology, pollination ecology and seed production of the ornamental plant *Salvia splendens* Sello. Journal of Horticultural Science and Biotechnology, 77(4): 498–501.

Şenol, S.G., Eroğlu, V., Şentürk, O., Kaçmaz, F., 2017. The pollination and reproduction success of *Salvia sclarea* Serdar. Biological Diversity and Conservation, 3: 130–135.

Shu, K., Liu, X.D., Xie, Q., He, Z.H., 2016. Two faces of one seed: Hormonal regulation of dormancy and germination. Molecular Plant, 9: 34–45.

Singh, M., Burson, B.L., Finlayson, S.A., 2007. Isolation of candidate genes for apomictic development in buffelgrass (*Pennisetum ciliare*). Plant molecular biology, 64(6), 673-682.

Song, Z., JianHua, W., HongGang, W., FuJuan, Z., LiWu, H., 2009. Studies of the floral biology, breeding characters of *Salvia miltiorrhiza*. Acta Horticulturae Sinica, 36: 905–910.

Subaşi, Ü., Güvensen, A., 2011. Breeding systems and reproductive success on *Salvia smyrnaea*. Turkish Journal of Botany, 35: 681–687.

Takano, A., 2013. Gynodioecy in *Salvia omerocalyx* Hayata (Lamiaceae). APG: Acta Phytota Geobot, 63(3): 149–153.

Wester, P., Claßen-Bockhoff, R., 2006a. Bird pollination in South African *Salvia* species. Flora, 201(5): 396–406.

Wester, P., Claßen-Bockhoff, R., 2006b. Hummingbird pollination in *Salvia haenkei* (Lamiaceae) lacking the typical lever mechanism. Plant Systematics and Evolution, 257: 133–146.

Wester, P., Claßen-Bockhoff, R., 2007. Floral diversity and pollen transfer mechanisms in bird-pollinated *Salvia* species. Annual of Botany, 100: 401–421.

Zapata, T.R., Arroyo, M.T.K., 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica, 10(3): 221-230.

خصوصیات گلدهی و سیستم اصلاح در هفت گونه مریم‌گلی (*Salvia* spp.) بومی ایران

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چکیده. پیش از شروع هر گونه برنامه اصلاحی در گیاهان، لازم است نسبت به رفتار باروری گیاه آشنایی کامل داشت. علیرغم تعداد زیاد گونه مریم‌گلی در فلور ایران، اطلاعات کمی در مورد سیستم اصلاحی این جنس وجود دارد. در مطالعه حاضر، خصوصیات گل و سیستم اصلاحی هفت گونه مریم‌گلی کشت شده در طی سالهای ۱۳۹۶ و ۱۳۹۷ مورد بررسی قرار گرفت. فنولوژی گونه‌ها و برخی از خصوصیات گل ثبت گردید و همچنین گرده‌افشانی دستی برای ارزیابی باروری گلها انجام گرفت. نتایج نشان داد، تمام گونه‌ها دارای دوره گلدهی طولانی (۱۴ تا ۴۱ روز به ترتیب در گونه *S. atropatana* و *S. virgata*) بودند. بیشترین گرده-افشانی توسط زنبورها و مگس‌ها صورت می‌گیرد. گونه *S. virgata* و *S. nemorosa* دارای حدود ۹۰٪ سازگاری با محل رویش جدید بودند. دو گونه *S. atropatana* و *S. syriaca* دارای گل‌های هترواستیل (خامه و پرچم دارای طول متفاوت) از نوع long-styled morph بودند. نتایج مربوط به گرده‌افشانی دستی نشان داد، بیشترین درصد تشکیل بذر در گرده‌افشانی آزاد با میانگین ۷۳/۶۳ بدست آمد. تشکیل بذر در تیمار ژینوگامی در گونه‌ها و جمعیت‌های مختلف در محدوده ۱۰/۲٪ (*S. syriaca*) تا ۳۲/۵٪ (*S. frigida*) بود. تفاوت در تشکیل بذر تیمارهای ژیتنوگامی (گرده افشانی بین دو گل در یک گیاه) و اتوگامی (خودگشنی) خودبخودی به ترتیب در محدوده ۱۷/۳۰-۲/۶۰٪ و ۱۲/۱۸-۲/۹۸٪ بود. نتیجه‌گیری،گونه‌های مریم‌گلی مورد مطالعه دگرگشن و نسبتاً خودناسازگار هستند. این گونه‌ها جهت باروری نیاز به حشرات گرده‌افشان‌کننده دارند و همبستگی بین میزان خودناسازگاری و ساختار هتروموروفی گل مشاهده نگردید.

کلمات کلیدی: بیولوژی گلدهی، خودناسازگاری، گرده‌افشانی‌دستی، مورفولوژی گل، هترواستیلی