Floral biology of medicinal plants II. *Lamiaceae* species

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Summary: Several species in the Lamiaceae family are therapeutically significant medicinal plants. Such as sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), peppermint (*Mentha piperita L.*). marjoram (*Majorana hortensis* Mönch), thyme (*Thymus vulgaris* L.), *Origanum spp.*, hyssop (*Hyssopus officinalis* L.), *Lavandula* spp., basil (*Ocimum basilicum* L.), lemon-balm (*Melissa officinalis* L.) are regularly produced in Hungary. Nevertheless, data on their floral biology are not satisfactory. This review intends to gather information on the characteristic constitution of flowers and inflorescences, blooming dynamics, pollination mechanism and crossability of some of the most significant medicinal and spice species

Flowers are mainly hermaphrodite, however, also monoeceous ones are turning up: pistillate flowers marjoram, tyme, oregano. In flowering of a plant individual, a basipetal blooming sequence is described. These species have a floral structure fully adapted to pollination by insects, and the majority of them is an excellent honey-plant (lavandel, hyssop, sage, etc.).

Flowers exhibit characteristic proterandry, therefore autogamy is considered to be almost impossible, and geintonogamy as well as xenogamy to be the most characteristic ways of fertilization. However, in several cases self-fertilization proved to be also successful. Crossability among cultivars or species is depending on the genotype combination and usually produces less seed than the above forms of fertilization.

Introduction

Medicinal plants in the *Lamiaceae* family represent mainly perennial plants and shrubs. Their dried flowering shoots or leaves are utilized as medicinal drug, having various therapeutical effects depending on the species. They accumulate essential oil in special oil ducts, contain tannins, organic acids and fatty oils. Antiseptic and antitussive effect of thyme, the sedative effect of lavandel and lemon-balm, the cholagogue and antispasmodic effect of peppermint, antioxidant activity of oregano or rosemary etc. are some of the most well known pharmacological directions. Marjoram, basil, hysopp, etc. are even better known as spices, while sage and lavandel may be conidered first of all as aromatic plants.They become more and more popular also as pot-herb.

In the last decade, it became more and more obvious, that world market demand may only be fulfilled if standard quality cultivars are commercially produced. An increasing breeding activity can be observed over the world. However, in breeding proper knowledge on reproduction biology including floral biology and fertilization aspects are consider to be essential. Gaps in the scientific background may lead to unsuccessful pollination, selfing, crossing, and other troubles. As these species have been produced at smaller surfaces usually, economics of the culture hardly allowed widespread research in this topics. The accumulated information might remain as practical knowledge for each breeder, while scientific publications are rather scarce. Therefore, it seems to be useful and filling certain gaps, if we summarize information in connection with flowering biology of some cultivated medicinal plant species of the *Lamiaceae* family.

Morphology of inflorescences

The species in the *Lamiaceae* family have a characteristic inflorescence constitution. The flowers are concentrated in a spike and individual flowers sit at the base of the leaves or are terminal, surrounded by bracts. Botanically, the inflorescence is an anthela with reduced spindle (*Dános,* 1997). Both the calyces and the petals are grown into tubes, but it is more pronounced in case of the corolla. The upper part often covers the stamina and the pistil. Usually there are four stamina, the fifth one is reduced. In the genera of *Salvia* and *Rosmarinus* the stamina are modified, only two of them hawe one fertile theca on the tip of an elongated connective, whereas the other end of the connective serves as a lifting arm. If insects touch the basal part, the opposite end moves down throwing pollen on their abdomens. This mechanism is characteristic also for medicinal plant species such as *Salvia officinalis* L., *Salvia sclarea* L., *Rosmarinus officinalis* L.

Boros (1968) supplies data on the morphology of the spikes of *Lavandula* species. Their inflorescence consists of

4–5 (rarely 3–8) pseudo-verticils, each of them having 6–10 flowers. Every verticil consists of two opposite dichasia. The most simple dichasium has 3, the bigger ones 5 flowers. The distance between pseudo-verticils on the shoot is growing into the basal direction.

Although the complete (bisexual) flowers are most general, in some species also female flowers appear. According *Heeger* (1956) in peppermint (*Mentha piperita L.*) and rosemary (*Rosmarinus officinalis* L.) beside the big proterandrous flowers smaller ones with lighter coloured corolla and degenerated stamina are to be found, too. There are no further data about the proved male sterility of these individuals. Peppermint usually does not produce any seeds, however, because of its hybrid origin. Occurrence of flowers having only female organ were mentioned also for sage (*Salvia officinalis* L.), (*Heeger,* 1956).

Pank et al. (1999) examined male sterility in 20 marjoram (*Majorana hortensis* Mönch) origins and found, that the rate of male sterility is characteristic for the accession, it varied between 2 and 100%. The male sterile plants could be visually distinguished according to their degenerated anthers. It was also established, that the male sterile plants exhibit a 3–15 days earlier flowering, than the fertile ones, but the grade of sterility is highly influenced by the weather. On the contrary, investigating several hundred individuals of basil, *Putievsky* et al. (1999) did not find any male sterile plant.

In thyme, (*Thymus vulgaris* L.) *Gouyon* et al. (1985) hypothesized a connection between sexual polymorphism and growing area, especially soil type. They found in the populations on limestone soil, – which was characterised as the xeric, "home" environment of tyme with very few disturbances – mainly hermaphrodite plants. In moist, deep, brown soils where several concurrent species are present, a high proportion of female plants was observed. It seems likely, that in the latter, more disturbed areas the constitution of the flowers determine higher rate of outcrossing and genetic diversity assuring higher viability for the population.

The opposite phenomenon was observed in oregano (*Origanum vulgare* ssp. *hirtum*). According to measurements of *Szabó* (unpublished data) in cultivated populations two types of plant individuals were present: ones with solely hermaphrodite flowers as well as plants with hermaphrodite and male flowers in a proportion of 55:45 %. The stamina hang out from flowers, and its direction is characteristic for intraspecific taxa of *Origanum* genus (*Ietswaart* 1980).

The morphology of the flowers of hyssop (*Hyssopus officinalis* L.) has been highly adapted to insect-pollination. The tube of the corolla is tightened from two side that only a small entrance remains to the nectaries. On the contrary, in case of marjoram, several insect species may fit into the corolla tube because it is short and wide (*Heeger,* 1956).

There are hardly any data about the size of the inflorescences. In case of hyssop for the length of inflorescences our measurements showed an average value of 12 cm with marginal values 6 and 18 cm over 6 years in 12 populations (unpublished data). It was proved, that the value changes according to genotype, ecological circumstances, age and health state of the plant. The number and size or even the existence of side-branches within the inflorescence is also very variable. According to *Ietswaart* (1980) the length of the spikes of *Origanum vulgare* ssp. *hirtum* is 3–35 mm, the length of the corolla 3–7,5 mm.

As for the colour of the petals, in the majority of the species a wide variability can be observed: different shades of white, pink, violet and blue appear.

Blooming dynamics

Blooming of a plant in case of *Lavandula* species lasts 3–4 weeks. Depending also on weather conditions, full bloom happens in 10–15 days after the opening of the first flowers (*Boros,* 1992). According to the mentioned author, the sequence of blooming time of lavandel species is the following: *L. angustifolia* MILL., *L. x intermedia* EMERIC., *L. latifolia* MEDIC. In the spikes of lavandel a strict sequence in opening of the individual flowers can be observed. In each dichasium the terminal flower is blooming as first one, after it the neighbouring flowers, followed by the marginal ones. The time shift among them assures, that the last ones open only when the terminal flower faded. A single flower is open for 2–3 days, however if no fertilization happens, it may last 12 days.

The described basipetal blooming sequence within a dichasium is similar in basil (*Ocimum basilicum* L.), (*Putievsky,* 1993). Also within an inflorescence, flowers start to bloom from the top of the shoot, further on at the side-branches into basal direction. According to data of *Szabó* et al. (1999) blooming of a basil plant may last in Hungary even 2 months.

As the different flowers open continuously within a spike, it is rather hard to define the blooming stages of a plant as a whole. For closer definition of blooming stages in basil *Szabó* et al. (1999) suggested a "flowering index formula", where coded developmental phases of individual flowers are taken into consideration. The development of individual flowers is divided into 8 stages from green bud stage till ripe seed stage. By the help of the flowering index, -to which a sigmoid function was fitted during onthogenesis-, the flowering stage of basil plant could be expressed at any actual moment. Growing conditions, especially water supply may influence blooming dynamics (*Aluri,* 1991).

The medicinal plant species of this family are characterized by proterandry, which is similar to the behaviour of *Apiaceae* species.

In marjoran, *Heeger* (1956) describes, that elongation of the pistil and its emergence from the corolla happens at the time, when the anthers dehisce. It was ascertained alaso by more recent investigations (*Fortunato & Ruta,* 1996).

Exact data on the fertile periods of stamina and pistil are published rarely. In *Mentha rotundifolia* and its hybrids *Yegorova & Bugaenko* (1988) established, that stigma

viability was maintained for 7–9 days, while maximum percentage of seed set was reached by pollination 3–4 days after opening of the flowers.

Pollination

Lamiaceae species have a floral structure adapted to pollination by insects (*Nation* et al., 1992). Most of the medicinal plant species of the *Lamiaceae* family are considered to be excellent honey-plants. Especially lavandel (*Lavandula* spp.), hyssop (*Hyssopus officinalis* L.), sage (*Salvia officinalis* L.) attract huge number of honey-bees, which fulfil the pollination in these stands (*Heeger,* 1956).

In Italy, *Ricciardelli & D'Albore*, examined pollinators of *Lamiaceae* species in detail. They established (1984) that for thyme honeybees are responsible for pollination in 97%. First of all honey-bees, accompanied by other *Hymenoptera* are found on marjoram and rosemary flowers too (*Heeger,* 1956). The common pollinators of *Ocimum* species are honey-bees and cabbage butterflies (*Pieris rapae*). The situation was the same by lavandel and hyssop, where beside honeybees, *Bombus* species, *Anthophora manicatum* were also found in higher quantities. Flowers of hyssop are also visited by species of *Diptera, Lepidoptera* genera as well *as Eristalis arbustotus, Syrphus balteatus.* Characteristic pollinators of *Origanum* spp. are wild insects, mainly *Syrphus* and *Musca* species (*D'Albore* et al., 1983/a).

Wild bees are abundantly (in 30–40%) visiting the flowers of savory (*Satureja* spp.), (*Ricciardelli & D'Albore,* 1988), while honey-bee is not a characteristic pollinator for this species (*Heeger,* 1956). Examining different mint species –including peppermint– and lemon-balm (*Melissa officinalis* L.) *D'Albore & Ricciardelli* (1983/b) determined *Syrphus* spp., *Andrena minutula, Anthidium manicatum* to be the most important pollinators.

Nation et al. (1992) supposed differences in pollinator preference among varieties of a species (*Ocimum basilicum*), however it could not be proved experimentally.

Fertilization properties

Based on the morphological constitution of the flowers and the mechanism of proterandry, *Lamiaceae* species are cross-pollinating ones. As no genetical background for self-incompatibility has been proved, beside xenogamy also geintonogamy may occur between flowers of the same plant. However, under certain circumstances autogamy within a single flower might take place, too. In lack of insect pollination, a spontaneous self-fertilization can occur in the flowers of lavandel (*Heeger,* 1956). The author does not exclude it also for hyssop. In our experiments carried out in the last ten years in Budapest, we produced seed by isolating inflorescences or parts of inflorescences in selected populations of hyssop, marjoram, sage and basil (unpublished results). Although seed-setting rates were low, it is proved, that self-fertilization of a plant individual is possible. The same results were found in Israel by *Putievsky* et al. (1999) for basil genotypes. They got 1–41% seed set in self-pollinated, isolated inflorescences, with high variance within and among accessions.

At the level of cultivars, data on compatibility are even more insufficient. Significant differences in the rate of outcrossing were proved among nine accessions studied by *Nation* et al. (1992). No difference was found, however, in outcrossing of early, middle and late blooming parts of the inflorescences. The estimated rates of outcrossing -using a purple leaf variety as marker- varied between 19.9% and 32.8% for five accessions and between 1.0% and 3.5% for four accessions. The authors declare further studies to be necessary for clearing whether low outcrossing rates are caused by self-pollination character of the accessions or by their special incompatibility with the marker parent. In hybridization studies of *Putievsky* et al. (1999) with *O. basilicum* accessions, they got 13–19% crossability (seed set) among them.

It is generally accepted, that *Mentha piperita*, as a natural hybrid does not produce any fertile seed. However, according *Himmelbaur & Hindes* (1928) cit. *Heeger* (1956) there are sometimes normal developed pollen grains as well as embryo-sacs to be found, which make pollination possible. In these cases the author found, that fertilization takes place only between cultivars or species, but never within the cultivar. Today it would be interesting to clear the current situation for peppermint, concerning the new chemotypes and varieties recently developed.

At the level of species, both xenogamy (fertilization within species) as well as bastardogamy (fertilization between species) may be successful, however, to a different rate. Artificial hybridization of sage was carried out by manual pollination after emasculation and isolation of the flowers (*Putievsky* et al., 1990). In different hybrid combinations of *S. fruticosa*, *S. tomentosa* and *S. officinalis* seed set was 2–34%. In comparison, seed set within the parent species was much higher, 85–96%.

In crossability between species, significant differences may arise depending on the direction of reciprocal crosses. It was proved also in the above experiment with sage, but it is described also for *Lavandula* species (*Boros*, 1992). While the hybridization of *L. latifolia* as maternal plant was successful with pollens of *L. angustifolia*, using the reciprocal combination no viable seed could be produced.

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