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Comparing nuclear DNA content, pollen viability, pollen production and seed retention of lavender and lavandin

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ABSTRACT

Lavender (*Lavandula* spp.) is one of the most widely grown essential oil crops in the world. This study aimed to determine the nuclear DNA contents using flow cytometry, pollen viability using TTC (2,3,5-Triphenyl Tetrazolium Chloride) and IKI (Iodine Potassium Iodide) tests, pollen production quantities using hemacytometric method, and seed retention rates per spike and flower of lavender (*L. angustifolia* var. Raya) and lavandin (*L. intermedia* var. Super) grown under ecological conditions in Isparta province of Turkey. The nuclear DNA contents were 2.11 and 2.54 pg $2C^{-1}$, respectively in the lavender and lavandin cultivar. The flowers of the lavender cultivar produced abundant pollen grains (average 5800 pollen per flower and 1450 pollen per anther) with high viability (60.65-65.05%) and seed retention rate per spike (91.57% on average). The lavandin cultivar, which had very low pollen viability (1.08-3.32%) and pollen grains (average of 2350 pollen per flower and 587.5 pollen per anther) gave very low seed retention rates per spike (0.60% on average). While each flower had four ovaries with the potential to produce four nutlets, lavandin flowers produced only trace numbers (0.15% on average) of seeds. As a result, the lavandin cultivar had more nuclear DNA content, longer stem and spike, smaller size but more numerous flowers, less and lighter anthers, lower pollen grains and viability, and vey few inviabile seeds compared to the lavender cultivar. It has been observed by eye that honey bees do not visit lavender and lavandin flowers for collecting pollen, but solely for collecting nectar.

1. Introduction

Lavender (*Lavandula* spp.), a semi-shrub and perennial plant from the Lamiaceae family, has a total of 39 species that are predominantly of Mediterranean origin and are distributed in 6 sections (*Lavandula, Dentatae, Stoechas, Pterostoechas, Subnudae*, and *Chaetostachys*) around the world (Upson and Andrews 2004). The most valuable commercially cultivated species, whose essential oils are utilized, are lavender (*L. angustifolia* Mill.), lavandin (*L. x intermedia* Emeric. ex. Loisel) and spike lavender (*L. latifolia* Medik.) in the *Lavandula* section (Beetham and Entwistle 1982). Among these, lavandin (syn. *L. hybrida*), also called "hybrid lavender", is a natural hybrid of *L. angustifolia* and *L. spica*, and was first identified in 1828 (Tucker 1985).

The essensial oils obtained by steam distillation of the lavender and lavandin fresh/dried flowers are rich in terpenic compounds such as linalool, linalyl acetate, and camphor, and have a wide range of uses including perfume, cosmetics, medicine, and food additives (Lawrence 1994). Compared to lavender varieties, lavandin varieties bloom later and have higher flower yield and oil content (Baydar 2022). However, lavender essential oil is more suitable for the perfumery industry due to its lower camphor and 1,8-cineole content and higher levels of linalyl acetate, lavandulyl acetate and lavandulol (Karık et al. 2017). The camphor contents of the essential oils obtained from Bulgarian lavender cultivars are below the upper limit value (<0.6%) required by ISO 3515:2002 standard (Stanev et al. 2016).

Both lavender and lavandin are cultivated in many countries of the world, especially in the Mediterranean and Balkan countries. The first commercial lavandin cultivation in Türkiye (Turkey) began in Kuyucak village in the Isparta province in the middle of the 1970s (Kara and Baydar 2011). Nowadays, Kuyucak village has created an agrotourism identity by attracting thousands of local and foreign tourists every year with the "The village with Lavandin Scent" and "Lavandin Festival" events and has become a role model for sustainable rural development (Tarhan 2020). *Lavandula* flowers which are purple in color, due to the pigments such as delphinidin and malvidin, and very fragrant due to the mono/sesquiterpenic essential oils, are of great value in terms of both ecotourism and beekeeping activities.

In recent years, lavender and lavandin production areas have reached up to 5000 hectares across Türkiye (TUİK 2023). Due to the lack of cultural practices such as irrigation, chemical fertilization and pesticide application, producers prefer *Lavandula* cultivation. Although lavandin (var. Super) is widely cultivated in the Western Mediterranean Region, lavender varieties (Bulgarian varieties such as Sevtopolis, Yubileina, Hemus, Hebar, Drujba, Raya and Karlova) are widely cultivated in other regions, especially in the Aegean, Marmara,

Mediterranean and Central Anatolian regions. An average of 7500 kg ha^{-1} fresh (with stems) and 1500 kg ha^{-1} dry (without stems) flowers are produced from the Super lavandin cultivar grown in Isparta province, and 1 kg of lavandin oil is obtained by steam distillation of 60-70 kg of fresh lavandin flowers (Baydar and Kineci 2009).

While lavandin cultivars, which have seed sterility, are only propagated vegetatively, lavender cultivars can be propagated both vegetatively and generatively (Urwin 2014). In addition, since lavender is a cross-pollinated plant, its seeds show a high degree of genetic variation, and seed-grown seedlings exhibit heterogeneous growth and development, as well as reaching economic yield age later (Baydar 2022). Due to these disadvantages, plantations are commonly established using rooted cuttings under *in vivo* and *in vitro* conditions (Kara and Baydar 2020). Although various opinions have been expressed as to why lavandin varieties show seed sterility, there is no exact data on this aspect (Beetham and Entwistle 1982). Therefore, it is necessary to understand the flowering, pollination, and fertilization biology of the lavender/lavandin plants for the plant breeding methods and the seed certification processes. The aim of this research was to compare the floral, pollination and fertilization characteristics of lavender and lavandin cultivars, in particular to obtain findings that will help to understand the problem of high seed sterility in lavandin scientifically.

2. Materials and Methods

This research was conducted at the Department of Field Crops, Faculty of Agriculture, Isparta Applied Sciences University in Türkiye. Lavender (*Lavandula angustifolia* Mill. var. Raya) and lavandin (*Lavandula x intermedia* Emeric ex Loiseleur var. Super) cultivars grown under ecological conditions in Isparta province were used as material in the flowering season of 2021.

2.1. Nuclear DNA content by flow cytometry

Since pollen and seed sterility in plants are closely, although not directly, related to the genome structure, the DNA contents of both varieties were determined. (Van Oost et al. 2021). The nuclear DNA content (pg 2C^{-1}) was determined using flow cytometry according to a protocol explained by Tuna et al. (2016). Healthy, young and fresh leaves of sample and standard plants were chopped in petri dishes containing 500 µL nuclei extraction buffer. The extract was passed through a 30 µm CellTrics filter, and transferred into a tube containing 2 mL staining solution (prepared by mixing 6 µL RNAse stock solution and 12 µL propidium iodide stock solution). The solution was incubated in the dark for 30-60 minutes before being read on a PARTEC Flow Cytometer device. The nucelar DNA content of the sample was calculated based on the relative positions of the G1 peaks of the sample and standard (*Vicia sativa* was used as the standard).

2.2. Pollen viability and productivity tests

During the flowering season of the cultivars (Raya bloomed from June 10 to July 15, 2021, and Super bloomed from June 25 to August 25, 2021), a sufficient number of mature flower buds were collected from the plants whose flowers were expected to open one day later. Anthers were separated from these flowers (each flower has 4 anthers) and spread over the petri dishes, which were kept at room temperature under a 60-watt lamp overnight. The pollen productivity of hexacolpat-structured (6-pore) pollen obtained from exploding anthers was tested using the hemacytometric method, and pollen viability was tested with 2,3,5-triphenyl tetrazolium chloride (2 hours in 1% TTC solution) and iodine-potassium iodide (1.5% in IKI solution) maximum 5 minutes) (Eti 1990 1991). For each cultivar, flower pollen grains were observed under a microscope (Nikon SE, 400×) using two slides, with four observations made on each slide for the TTC test, which stained the grains dark red, and the IKI test, which stained them dark brown as seen in Figure 1. Pollen grains that stained dark red or dark brown were considered viable, while those that remained unstained or appeared yellow/brown were considered non-viable (Norton 1966). Additionally, the width and length (in µm) of one hundred randomly selected pollen grains were measured during pollen viability tests.

Mature anthers of 10 flowers were used to determine the average pollen amount (number) per anther by the hemocytometric method. Forty anthers were soaked overnight in 3 ml of distilled water to which one drop of Tween-20 was added. Later, a suspension containing a drop of pollen was placed onto each of the two counting chambers on a hemocytometric slide and covered with a coverslip. Under a microscope (Nikon SE), the flower pollen grains, in four randomly selected large squares, were counted in each counting chamber. Using the volume of the space between the hemocytometer slide and coverslip (0.1 mm^3) and the total number of pollen grains in a 3 ml suspension (Eti 1990), the average number of pollen grains per anther was determined by dividing the values obtained by 10, and the number of pollen grains per anther was multiplied by 4 to determine the average number of pollen grains per flower.

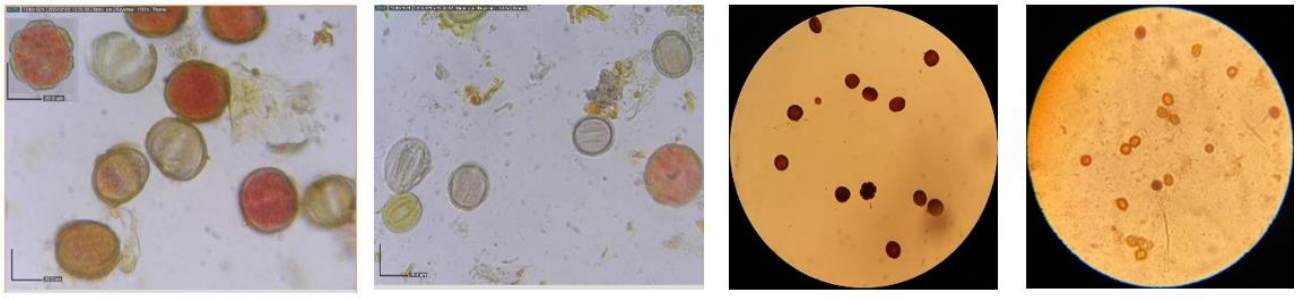

Figure 1. Pollen viability tests of lavender (var. Raya) and lavandin (var. Super).

2.3. Floral and scent charactersitics

Various parameters explained by Kara (2011) were determined for each cultivar including flower stem height (cm), spike length (cm), number of nodes per spike, number of flowers per node, number of flowers per spike, flower width and length (mm), anther width and length (µm), anther weight (g 1000 flowers^{-1}), seed retention (setting) rate per spike [(total number of seeds/total number of flowers) x100], seed retention rate per flower [(number of seeds/number of ovaries) x100], 1000-seed weight (g), and seed germination rate (%). Since lavender plants use the attractive properties of scents to attract pollinator insects (Benachour 2017; Valchev et al. 2022), the essential oil contents of both lavender species were determined. At the full flowering stage, the essential oil content (%) of fresh flower with stem was determined by water distillation for 3 hours using a Clevenger apparatus (Kara and Baydar 2011, 2012, 2013).

2.4. Statistical analysis

The data obtained from the examined characteristics of the cultivars were presented as mean ± standard deviation, and the significance of the differences between means was checked using the Student t-test (SAS 1998).

3. Results

The average nuclear DNA contents measured in triplicate on fresh leaf samples taken from each species and flow cytometry histograms of the lavender and lavandin cultivars are shown in Figure 2. The sample peaks were clearly distinguishable from the standard plant G1 peak. The nuclear DNA contents were 2.11 ± 0.05 pg 2C^{-1} and 2.54 ± 0.08 pg 2C^{-1}, respectively in the lavender (var. Raya) and lavandin (var. Super) cultivar (Figure 2). The floral characteristics of lavender (var. Raya) and lavandin (var. Super) are presented in Table 1. Statistically significant differences were found between the cultivars Raya and Super in terms of flower stem length (31.80 ± 8.63 and 61.00 ± 6.16 cm), spike length (11.40 ± 2.27 and 15.10 ± 3.25 cm), number of nodes per spike (5.50 ± 0.53 and 10.20 ± 0.79), number of flowers per node (7.90 ± 1.45 and 18.10 ± 2.56), number of flowers per spike (43.20 ± 7.64 and 184.50 ± 29.70), pollen width (37.64 ± 2.01 and 25.31 ± 1.40 µm) and length (41.81 ± 3.18 and 27.18 ± 1.58 µm), pollen viability (60.65 ± 11.31 and 1.08 ± 0.85 in TTC test, 65.05 ± 21.51 and 3.32 ± 1.95 in IKI test; Figure 1), pollen production (1450 ± 287.9 and 587.5 ± 356.1 per anther), seed retention rate per spike (91.57% ± 27.68 and 0.60% ± 0.27), seed retention rate per flower (22.89% ± 6.92 and 0.15% ± 0.07), and essential oil content (1.25% ± 0.10 and 1.75% ± 0.25), respectively (Table 1). However, no statistically significant differences were observed between both varieties in terms of flower width (2.45 ± 0.25 mm and 2.30 ± 0.15 mm) and length (12.05 ± 2.00 mm and 11.68 ± 1.70 mm), anther width (0.60 ± 0.10 mm and 0.50 ± 0.10 mm), anther length (1.00 ± 0.10 mm and 0.80 ± 0.15 mm), anther weight (0.08 ± 0.01 g and 0.07 ± 0.01 g 1000 pcs^{-1}), and 1000 seed weight (1.15 ± 0.12 g and 0.09 ± 0.17 g, respectively (Table 1).

4. Discussion

According to the results of the flow cytometer analysis, it was determined that the nuclear DNA amount was 2.11 pg 2C^{-1} in the lavender cultivar and 2.54 pg 2C^{-1} in the lavandin cultivar (Figure 2; Table 1). Indeed, in a comprehensive karyotype study conducted on a total of 82 genotypes in the *Lavandula, Stoechas, Dentatae, Pterostoechas,* and *Subnudaeda* sections of lavender, it was reported that the genome size ranged from 0.76 to 4.80 pg 2C^{-1} and the chromosome numbers varied between 22 and 100 (Van Oost et al. 2021). In the same study, it was explained that the 2*n* chromosome number of *L. x intermedia* 'Heavenly Angel' genotype was 100. The chromosome numbers of species belonging to *Lavandula* subsection have been determined as 2*n*= 34, 36, 42, 48, 50, 54, and 75 (Garcia 1942; Darlington and Wylie 1955; Upson 2004; Rice et al. 2015). It has been reported that there is a close relationship between the genome size (2C values) and chromosome number of the species in this section, and that those with high nuclear DNA amounts also have a higher number of chromosomes (Van Oost et al. 2021).

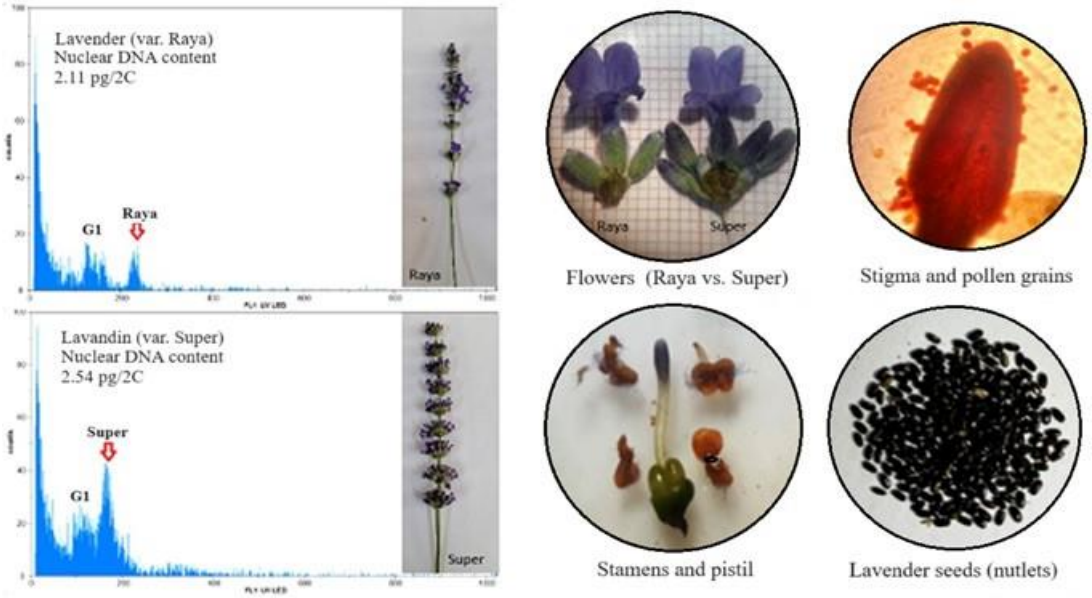

Figure 2. Flow cytometer histograms and generative reproductive organs of lavender (var. Raya) and lavandin (var. Super).

Table 1. Nuclear DNA contents and floral characteristics of lavender (var. Raya) and lavandin (var. Super)

Characteristics	Lavender (var. Raya)	Lavandin (var. Super)	t- value
Genome size			
DNA content (pg $2C^{-1}$)	2.11 ± 0.05[a]	2.54 ± 0.08	
Flower properties			
Flower stem height (cm)	31.80 ± 8.63	61.00 ± 6.16	8.70**
Spike length (cm)	11.40 ± 2.27	15.10 ± 3.25	2.95**
Number of nodes per spike	5.50 ± 0.53	10.20 ± 0.79	15.67**
Number of flowers per node	7.90 ± 1.45	18.10 ± 2.56	10.97**
Number of flowers per spike	43.20 ± 7.64	184.50 ± 29.70	14.57**
Flower dimensions			
Flower width (mm)	2.45 ± 0.25	2.30 ± 0.15	1.49
Flower length (mm)	12.05 ± 2.00	11.68 ± 1.70	0.44
Anther dimensions			
Anther width (mm)	0.60 ± 0.10	0.50 ± 0.10	0.59
Anther length (mm)	1.00 ± 0.10	0.80 ± 0.15	0.68
Anther weight (g 1000 pcs^{-1})	0.08 ± 0.01	0.07 ± 0.01	0.17
Pollen dimensions			
Pollen width (400x) (µm)	37.64 ± 2.01	25.31 ± 1.40	17.44**
Pollen length (400x) (µm)	41.81 ± 3.18	27.18 ± 1.58	14.27**
Pollen production			
Pollen number per anther	1450 ± 287.9	587.5 ± 356.1	8.42**
Pollen number per flower	5800 ± 1151.7	2350 ± 1424.4	8.42**
Pollen viability			
TTC test (%)	60.65 ± 11.31	1.08 ± 0.85	23.43**
IKI test (%)	65.05 ± 21.51	3.32 ± 1.95	12.78**
Seed fertility			
Seed retention rate per spike (%)	91.57 ± 27.68	0.60 ± 0.27	10.39**
Seed retention rate per flower (%)	22.89 ± 6.92	0.15 ± 0.07	10.39**
1000 seed weight (g)	1.15 ± 0.12	0.09 ± 0.17	0.79
Essential oil content (%)	1.25 ± 0.10	1.75 ± 0.25	2.49*

[a]All values are means ± standard deviation (n= 10). Asterisk marks significant differences (*$P \leq 0.05$ and **$P \leq 0.01$) between lavender and lavandin for that parameter according to Student's t-test.

Since lavandin (*L. x intermedia*) is a natural hybrid between lavender (*L. angustifolia*) and spike lavender (*L. latifolia*), it would be expected to have an intermediate 2C value between the amounts of their two ancestral species. Indeed, in the karyotype study conducted by Van Oost et al. (2021), which confirms this view, genome sizes were determined as 2.02-2.25 pg $2C^{-1}$ in *L. angustifolia* (2*n*= 48), 2.48-2.59 pg $2C^{-1}$ in *L. latifolia* (2*n*= 50), and 2.28-2.37 pg $2C^{-1}$ in *L. x intermedia* (2*n*= 50). However, in our findings, while the 2C values of *L. angustifolia* were similar, the 2C DNA amount of *L. x intermedia* was found to be close to the value of *L. latifolia*, not between the values of *L. angustifolia* and *L. latifolia* determined by Van Oost et al. (2021) (Table 1). Therefore, more detailed karyotype analyses and molecular phylogenetic research are needed to dispel doubts on the *Lavandula* species.

While lavandin produces little to no seeds due to its sterility, lavender and spike lavender which are the ancestral species of lavandin are fertile and produce seeds (Tucker 1985). In our study, it was found that the lavender cultivar produced a large number of pollen (an average of 5800 grains per flower) with high viability (60.65% to 65.05%). However both pollen viability (1.08% to 3.32%) and pollen production (an average of 2350 grains per flower) were found to be relatively lower in the lavandin cultivar (Table 1). Due to the very low pollen productivity and viability, it was observed that the seed setting (retention) rate of the lavandin variety was very low (average of 0.6% per spike). Additionally, lavender seeds germinated on average at a rate of 35% in pure water and 50% in 0.1% GA_3 solution within a week (not tabulated).

Due to the lack of regular pairing between homologous chromosomes during meiosis in the lavandin genome, which has different chromosome numbers from its parents, gametes with aneuploid chromosome numbers that can cause infertility or gametophytic sterility have been formed. Pollen sterility and seed sterility are also widespread in English mint, such as lavandin. English mint (*Mentha x piperita*, 6x= 72), which is a natural hybrid of water mint (*M. aquatica*, 8x= 96) and garden mint (*M. spicata*, 4x= 48), is an allohexaploid species (Tucker 2012).

However, instead of producing 6x= 72 (48+24) chromosome offspring through normal meiotic fertilization, different ploidy levels such as 48, 60, 72, 84, and 96 (cytomixis) can occur (Tucker and Fairbrothers 1981).

Each lavender or lavandin flower carries 4 male organs (stamens) and 1 female organ (pistil) with 4 ovaries (Figure 2) with a potential to produce 4 nutlets (seeds). However, it was observed that these four ovaries could produce about one seed (22.89% on average) in lavender, and trace number of seeds (0.15% on average) in lavendin flower. It has been determined by Urwin (2014) that autotetraploid lavandin (var. Grosso and Seal) plants with doubled chromosome numbers through colchicine application were able to become fertile and produce seeds.

In our study, the lavandin cultivar has shown narrower but longer leaves, longer stems and spikes, smaller but more numerous flowers, smaller and lighter anthers, pollen and seeds compared to the lavender cultivar. As a result of the "gigas effect" that occurs parallel to the increase in chromosome number and nuclear DNA amount in polyploid plants, there are increases in the size of organs such as leaves, flowers, fruits, and seeds, as well as the numbers of stomata and chloroplasts, and the amounts of secondary metabolites (Simmonds 1980; Sattler et al. 2016; Eng and Ho 2019). At least for now, based on the scientific findings and interpretations, it is believed that lavandin is not a polyploid species but rather a natural hybrid of lavender and spike lavender. Moreover, it is still a matter of curiosity whether lavandin shows the gigas effect since it has a higher chromosome number and nuclear DNA amount than lavender. The findings obtained from these studies are valuable at least in terms of providing insights on this issue.

Lavender flowers are pollinated, especially by bumble and honey bees (Valchev et al. 2022). However, we observed in this study that honey bees (*Apis mellifera*) visit lavender and lavandin flowers only to collect nectar, not pollen. The honey bee lands on the narrow flowers that are too small to fit in, and sucks nectar from the 6-7 mm long tongue through the 7-8 mm long corolla tube in an average of 2.32 seconds, only transferring pollen by contact with the fuzzy front of its head to the anthers (Benachour 2017). Although the bees help with pollination by carrying pollen on their bodies, their contribution to pollination is lower in lavandin flowers, which produce approximately three times less pollen than lavender flowers.

5. Conclusion

As a result, the lavandin genome contains a higher amount of nuclear DNA than lavender. Lavandin also blooms later, forms a wider and taller habitus, produces longer flower stems and spikes, and yields more flowers. Finally, it contains a higher percentage of essential oil. However, due to low pollen viability and production, its seed-setting rate remains very low in lavandin compared to lavender. The findings from this research will be a valuable tool for evaluating the opportunities and possibilities of the lavender and lavandin breeding approaches involving hybridization.

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References

Baydar H, Kineci S (2009) Scent composition of essential oil, concrete, absolute and hydrosol from lavandin (*Lavandula x intermedia*). Journal of Essential Oil Bearing Plants 12(2): 131-136. doi: 10.1080/0972060X.2009.10643702.

Baydar H (2022) Science and Technology of Medicinal and Aromatic Plants. 10th Edition, Nobel Academic Publishing, Ankara, Turkey.

Beetham J, Entwistle T (1982) The Cultivated Lavenders. Royal Botanic Gardens, Melbourne.

Benachour K (2017) Insect visitors of lavender (*Lavandula officinalis* L.). African Entomology 25: 435-444. doi: 10.4001/003.025.0435.

Darlington C, Wylie A (1995) Chromosome Atlas of Flowering Plants. G. Allen & Unwin Ltd., UK.

Eng WH, Ho WS (2019) Polyploidization using colchicine in horticultural plants: A review. Scientia Horticulturae 246: 604-617.

Eti S (1990) Çiçek tozu miktarını belirlemede kullanılan pratik bir yöntem [A practical method used to determine the amount of pollen.]. Çukurova Üniversitesi Ziraat Fakültesi Dergisi 5(4): 49-58.

Eti S (1991) Bazı meyve tür ve çeşitlerinde değişik *in vitro* testler yardımıyla çiçek tozu canlılık ve çimlenme yeteneklerinin belirlenmesi. Çukurova Üniversitesi Ziraat Fakültesi Dergisi 6(1): 69-80.

Garcia J (1942) Contribution towards the karyo-systematic study of the genus *Lavandula* L. Boletim da Sociedade Broteriana 13: 183-193.[In Portuguese].

Kara N (2011) Uçucu yağ üretimine uygun lavanta (*Lavandula* sp.) çeşitlerinin belirlenmesi ve mikroçoğaltım olanaklarının araştırılması. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, Isparta.

Kara N, Baydar H (2011) Türkiye'de lavanta üretim merkezi olan Isparta ili Kuyucak yöresi lavantalarının (*Lavandula x intermedia*) uçucu yağ özellikleri. Selçuk Tarım ve Gıda Bilimleri Dergisi 25(4): 41-45.

Kara N, Baydar H (2012) Essential oil contents and composition of lavenders and lavandins cultivated in Turkey. Research on Crops 13 (2): 675-681.

Kara N, Baydar H (2013) Determination of lavender and lavandin cultivars (*Lavandula* sp.) containing high quality essential oil in Isparta, Turkey. Turkish Journal of Field Crops 18(1): 58-65.

Kara N, Baydar H (2020) The foral and scent characteristics of lavandin from the micropropagation and cutting propagation. Turkish Journal of Field Crops 25: 114-121.

Karık Ü, Çiçek F, Çınar O (2017) Menemen ekolojik koşullarında lavanta (*Lavandula* spp.) tür ve çeşitlerinin morfolojik verim ve kalite özelliklerinin belirlenmesi. Anadolu Journal of Aegean Agricultural Research Institute (AARI) 27(1): 17-28.

Lawrence BM (1994) Essential Oils. Allured Publishing Corp., Wheaton, IL, Perf. Flav. 19, 33.

Norton JD (1966) Testing of plum viability with tetrazolium salts. Proc. The American Society for Horticultural Science 89: 132-134.

Rice A, Glick L, Abadi S, Einhorn M, Kopelman N, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The chromosome counts database (CCDB) - A community resource of plant chromosome numbers. New Phytologis 206: 19-26.

SAS (1998) SAS Introductory Guide, 3rd Edition, NC, USA, pp. 99.

Sattler MC, Carvalho CR and Clarindo WR (2016) The polyploidy and its key role in plant breeding. Planta 243: 281-296.

Simmonds NW (1980) Polyploidy in plant breeding. The Journal of the South Pacific Association for Commonwealth Literature and Language Studies (SPAN) 23: 73-75.

Stanev S, Zagorcheva T, Atanassiov I (2016) Lavender cultivation in Bulgaria-21st century developments, breeding challenges and opportunities. Bulgarian Journal of Agricultural Science 22: 584-590.

Tarhan Y (2020) Kırsal kalkınma kapsamında lavanta tarımı-ekoturizm ilişkisi: Isparta ili Kuyucak köyü örneği. M.Sc. Thesis, Bartın University, Graduate School of Natural and Applied Sciences Bartın.

Tucker AO (1985) Lavender, Spike, and Lavandin. The Herbarist 51: 44-50.

Tucker AO (2012) Genetics and breeding of the genus *Mentha*. Journal of Medicinally Active Plants 1(1): 19-29.

Tucker AO, Fairbrothers DE (1981) An euploid series in an F_1 interspecific hybrid progeny of *Mentha*. Bulletin of the Torrey Botanical Club 108: 51-53.

TUİK (2023) Turkish Statistical Institute, Crop Production Statistics. https://biruni.tuik.gov.tr. Accessed 25 January, 2024.

Tuna GS, Keleş H, Göçmen D (2016) Flow sitometri ile çok yıllık buğdaygil yem bitkisi genetik kaynaklarının karakterizasyonu [Characterization of genetic resources of perennial forage grasses by using flow cytometer]. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi 25: 7-12.

Upson T, Andrews S (2004) The Genus Lavandula. Royal Botanic Gardens Kew: Richmond, UK.

Urwin NAR (2014) Generation and characterisation of colchicine-induced polyploid *Lavandula* × *intermedia*. Euphytica 197: 331-339. doi: 10.1007/s10681-014-1069-5.

Valchev H, Kolev Z, Stoykova B and Kozuharova E (2022) Pollinators of *Lavandula angustifolia* Mill. BioRisk 17: 297-307.

Van Oost E, Leus De Rybel B, Van Laere K (2021) Determination of genetic distance, genome size and chromosome numbers to support breeding in ornamental *Lavandula* species. Agronomy 11: 2173. doi: 10.3390/agronomy11112173.