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Induced polyploidization in medicinal species of the Lamiaceae family: A Review

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Declaration

I hereby declare that I have done this thesis entitled “Induced polyploidization in medicinal species of the Lamiaceae: A Review”, independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague date

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Zuzana Rázková

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Abstract

Medicinal plants and humanity have been growing side by side since immemorial times. The knowledge of use of plants spread according to people's traditions and was transmitted from one generation to another. With the knowledge, grew the desire for an enhancement of plant materials, as the improvement of soil elements by fertilization or selection of better varieties of species. Nowadays there are faster and more effective technologies helping to increase the chemical properties of the plant, as the polyploidization.

This review compared 17 plant species from 19 different experiments where the induced polyploidization was performed. All of these species were from Lamiaceae family containing components which are source of pharmaceutical substances. These plants were compared according to their plant part, method of induction, used antimitotic agent, morphological and chemical changes. Their chemical changes were after linked with their health use.

The most effective analysed parameters were nodal segments, in vitro cultivation and inductive medium. The use of antimitotic agent efficiency could not be reviled since colchicine is used in most of the reviewed experiments. Most of the compare plants increased size of stomata and decreased their frequency. Weight growth, larger thickness and darker colour were other characteristics recognised the most in polyploid plants. The increase of body surface reflected also in the increase of the increase of chemical compounds in plant's body in overwhelming majority of plants. The compounds changes could increase the anti-inflammatory, antioxidant, anticancer, antidiabetic, antimicrobial effects and treating many disorders cardiovascular, nervous or digestive systems and used for many other goods.

Key words: Lamiaceae, medicinal plants, polyploidization, secondary metabolites

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1. Introduction

Herbal medicine and humankind have been evolving next to each other since the beginning of our time and distinctive the exact moment when people started to use plants for medical purposes is nearly impossible. Nevertheless, the science of medicinal plants took place in one of the oldest civilisations in China, Greece, Egypt or India, and the oldest documented script gave a date about 5000 years ago and even more far over 6000 years ago also goes the evidence that people were cultivating medical plants (Jamshidi-Kia et al. 2018).

Centuries ago, people did not know anything about the composition of plants nor their right usage; for this reason, they had to rely on their intuition and mostly on their experience to use plants for medicinal purposes. The knowledge was transferred from one generation to another, and it was also developed and improved (Kumar Srivastava 2018).

Nowadays, people individually do not depend on the knowledge acquired in the past by their families about herbal healing, and most of them do not realise how much their lives and health rely on medical substances from plants. However, nearly 80 % of the population depends on medical help from plant sources (Máthé 2015). This behaviour displays growth in medicinal plants' use over time; for example, during the Palaeolithic period there is a record that over 8000 species were used for curative drives (Huang 2011). Currently, more than 50000 species of plants are used for medical purposes, and by using new technologies, it is even more likely to find every day new healing species (Jamshidi-Kia et al. 2018).

The technologies did not come only for finding new kinds of plants but also for improving the potential of the once we already knew and are likely used in the medicinal area. One type of technique is called polyploidy, which duplicates the genetic information, change its morphological, physiological or chemical structure of the plant, and then possibly the number of compounds used in the pharmaceutical industry (Fox et al. 2020).

Yet, some plants already in nature contain more amounts of medicinal compounds; therefore, these plants stand in the first stages of polyploidy induction for these purposes (Salma et al. 2017). One family like this is Lamiaceae, with a high content of essential oils, which hold the healing properties (Niazi et al. 2019).

Due to improvement and increase of efficiency in induced polyploidy among plants with healing effects from the Lamiaceae family, this thesis focuses on comparing all published and described species from this family on which have been performed polyploidy induction (*in vitro* or *ex vitro*). This research would benefit the plant breeding of the Lamiaceae family and provide a more efficient polyploidization process for the upcoming experiments.

2. Aim of the Thesis

The thesis aim was to perform a literature review using several references from different scientific databases to describe and determine more efficient methods on the induced polyploidization in medicinal species of the Lamiaceae family.

3. Methodology

This thesis was carried out as a review, with the use of academical literature. Data were collected from various databases like Science Direct, Web of Science, Scopus, Google scholar, Taylor & Francis, Nature or SpringerLink and updated until the recent year 2021. For research were used main keywords Lamiaceae family, polyploidy, medicinal plants, induced polyploidy, autoploidy, secondary metabolites, essential oils, antimitotic agents.

The information and data gained from articles were more explored, and they were summarised in Microsoft Excel file and sorted into clear table and explained in text.

4. Literature review

4.1. Medicinal plants

According to an article published by the World Health Organization, three of the deadliest diseases in the past twenty years are ischaemic heart disease, stroke, and lower respiratory infections (HWO 2020). All of these three diseases or their symptoms can be treated or prevented by medicinal plants (Adesina et al. 2017; Guo et al. 2018b; Gaire 2018) but plant therapy is efficient in many other diseases.

The term medicinal plant is used for plants constituted by biologically active substances that cause a healing effect in people's bodies. They can prevent and cure illnesses or affect and change a particular physiological function. An active substance, like essential oil, is mainly found in secondary metabolites, intermediates and products of metabolism (Máthé 2015; Tiwari & Rana 2015).

4.1.1. Secondary metabolites

Whereas primary metabolites are directly connected to the plant living cycle, growth, development or reproduction, for many years, secondary metabolites were considered a waste product with no specific use for a plant (Ashraf et al. 2018).

Today, it is known that the secondary metabolites hold the leading role in ensuring that the plant will have a higher chance of survival and that they are not only connected with simple metabolic processes in the cells. These naturally occurring substances are produced to respond to biotic agents like bacteria, viruses, and fungi, and abiotic factors such as temperature extremes, light radiation, or chemicals taken from soils. Their primary purpose is to protect the plant from potential enemies, they help the plant with its reproductive cycle, and it also helps the plant adapt itself to the environment (Ashraf et al. 2018; Yang et al. 2018).

In the plant kingdom are more than 100000 compounds of secondary metabolites; henceforth, each plant holds a different number and different kinds (Zaynab et al. 2018). They can also be separated into three main groups: Nitrogen-containing, and phenolic compounds and terpenes.

The biosynthesis of nitrogen-containing compounds is usually connected with the presence of some number of amino acids as tryptophan, tyrosine, and lysine. These compounds are widespread in the pharmaceutical industry and responsible, for example, for cough analgesic and suppressant effects of *Papaver somniferum* used in morphine or codeine. The second group, phenolic compounds, is the essential and aromatic group composed of two main subgroups, flavonoids, and phenylpropanoids. They are in charge of reproduction, growth, and resistance against stresses (Ashraf et al. 2018). Terpenes is the last and the biggest group of all secondary metabolites. They are based on natural hydrocarbon products with structure derivates from isoprene. These compounds support pollination by beatless and help plants to produce some of their hormones (Pagare et al. 2015; Tiwari & Rana 2015).

For people, the secondary metabolites are the most significant source of medical substances, and they can be determinate by colours, smell, or taste (Tiwari & Rana 2015; Ashraf et al. 2018). To obtain secondary metabolites in several species can be through essential oils (Valdivieso-Ugarte et al. 2019).

4.1.1.1. Essential oils

The essential oils are obtained by water-steam distillation from different plant parts, but mainly, this process is carried out from the blooms, leaves or stems. They are aromatic, hydrophobic and volatile liquids based on secondary metabolites (Valdivieso-Ugarte et al. 2019).

The essential oils are distinctive by their robust smell properties utilised in culinary or cosmetic areas (Sarkic & Stappen 2018), but they are also recognised for their bioactive compounds, which keeps healing effects. The use of the essential oils differs according to the biochemical composition; they hold antioxidant, anti-inflammatory, antimicrobial, antibacterial effects and even more (de Lavor et al. 2018; Tariq et al. 2019).

The oils are usually located in nondifferentiated cells or organs with secretion, such as secretory ducts in Mirthaceae or in granular hairs of the epidermis in Lamiaceae as an example (Ríos 2016).

4.2. Lamiaceae family

The Lamiaceae family, often called a Mint family, is present in all the continents and in all different kinds of climates. It can be found mainly in Mediterranean regions and Southwest Asia. The Lamiaceae holds around 235 genera and more than 7,000 species (Xu & Chang 2017).

4.2.1. Classification

Kingdom:	Plantae
Division:	Angiosperme
Class:	Dicotyledonae
Sub-class:	Gamopetalae
Order:	Lamiales
Family:	Lamiaceae

4.2.2. Morphological structure

The Lamiaceae contains annual or perennial herbs, semi-shrubs, shrubs or trees, which are typically squared shaped erected stamp with leaves most often simple, opposite and decussate (Raja 2012). They belong to the division Angiosperms; thus, the flowering bloom is also one of their characteristics. Flowers are bisexual, zygomorphic, sometimes unisexual and actinomorphic. They grow in shorter verticillasters formed into two –lipped mouth crown with usually four dioecious stamens (Xu & Chang 2017).

4.3. Medicinal plants from the Lamiaceae family

The Lamiaceae is one of the widest families used in traditional medicine and in the pharmaceutical industry. This is because the plants belonging to this family have essential oils as relevant ingredients, which can be differently applied in diverse areas of the health sector (Venkateshappa & Sreenath 2013). They could be used as antioxidants, help with nausea, headaches, anxiety, protect from multiple kinds of cancers, and even more; however, all their uses depend on their species.

The most known species from the Lamiaceae family are the *Salvia officinalis* L., *Mentha piperita* L., *Thymus vulgaris* L., *Lavandula officinalis* L., and *Rosmarinus officinalis* L. (Srancikova et al. 2014). This is just a small part of the Lamiaceae, many other plants are used in traditional medicine, and a great amount is on their way to be recognised for their benefits (Nieto 2017).

4.3.1. Most used aromatic plants from Lamiaceae family

The genus *Salvia* with nearly 1000 species, it is the largest genus in Lamiaceae (Bisio et al. 2019). It often contains strong aromatic herbs as *Salvia officinalis* L. (see Figure 1). The sage is originally from the Mediterranean and Middle East areas, here it took a rightful place in the local cuisine and folk medicine, and from this place, it was spread worldwide (Ghorbani & Esmaeilizadeh 2017). The most bioactive components involved in the healing process comprised in the Sage are the terpenes, phenolic acids, and flavonoids.

Rosmarinic acids, luteolin, apigenin, scutellarein, sagerinic acid are the highest containing compounds in the *Salvia officinalis* (Afonso et al. 2019).

The rosmarinic acid, luteolin and apigenin can be used in various pharmacological fields. All of them have antioxidant effects with other substances as carnosol, caffeic acid, quercetin, rutin, and others (Ghorbani & Esmaeilizadeh 2017). The rosmarinic acid and apigenin have been studied for their ability to inhibit the growth of cancer cells in various parts of the human body (Imran et al. 2020).

Moreover, the *Salvia officinalis* has even more goods; it is a plant, which thanks to its essential oils has antiviral and antibacterial effects, anti-inflammatory and antinociceptive effects, immunostimulating, metabolic, and memory-enhancing effects (Mansourabadi et al. 2016).

When it is spoken about anti-inflammatory and antinociceptive effects, it is mandatory to speak about the group flavonoids and terpenes, salvigenin, manool, carnosol, and urolic acid (Abad et al. 2011; El Euch et al. 2019).

Figure 1: *Salvia officinalis* L.

Source: Ghorbani & Esmaeilizadeh (2017)

Another plant widely used is *Thymus vulgaris* L. (Thyme) depicted on Figure 2, known for its diverse gastronomic uses but also, it acquired a top place in traditional medicine applications (Nikolić et al. 2014). The Thyme is recommended for its antimicrobial, antiviral, antiseptic, anti-inflammatory, anticancer properties, and in the fresh form, it has one of the highest antioxidant levels within all the herbs (Dauqan & Abdullah 2017).

Figure 2: *Thymus vulgaris* L.

Source: Klementa (2012)

It contains mainly phenolic and flavonoids compounds as zeaxanthin, lutein, apigenin, naringenin, luteolin, thymonin, thymol, carvacrol, eugenol, linaloon, even more (Dauqan & Abdullah 2017).

Thymol is the main phenolic compound, the major oil compound in *Thymus vulgaris,* the most important essential oil in this plant and it is widely used for its antioxidant properties. However, another source of antioxidants within this specific specie could be zeaxanthin, lutein, and luteolin (Gedikoğlu et al. 2019). Aside from its antioxidant effects, the Thymol also offers antiseptic, antibacterial, and antifungal assets.

Lavandula officinalis L., shown on the Figure 3, as the herbs already mentioned, is also well known for its distinguishing aromatic properties; name in Latin *lavo, lavare* means clean and that is why the Lavandula is used in many kinds of cosmetic products like soaps (Prusinowska & Śmigielski 2014). The Lavadnula is rich in bioactive components like linalool, linalyl acetate, lavandulol, 1,8-cineole, lavandulyl acetate, and camphor. Nonetheless, the linalool holds up one-third of all the plant components (Hassanpouraghdam et al. 2011). The Linalool has anti-inflammatory and analgesic substances, and with other elements, it can be used as a therapeutical agent (Cavanagh & Wilkinson 2005); for instance, in Iran is traditionally also used in memory dysfunction issues (Rabiei et al. 2014).

Figure 3: *Lavandula officinalis* L.

Source: Flóra (2021)

The *Rosmarinus officinalis* L. with the common name Rosemary (see Figure 4) as it is visible from the plant's name, is recognised for the rosmarinic acid, but the rosemary is not only known for the acid's richness, but it is also broadly used in cookery and medicine. It is composed of flavonoids, di and triterpenoids, monoterpenes, sesquiterpenes, alcohol, ester, ketone, hydroxycinnamic derivatives and other minor derivatives (Andrade et al. 2018; Aitfella Lahlou et al. 2021).

Even when the plant's name could indicate something different, the most involved compound is carnosic acid which can be as high as 30 % of bioactive compounds. The carnosic acid is in the group of terpenes, having a high impact on human health, it is an antiproliferative, antitumor, anti-inflammatory substance, and it answers for the protective effect of photoreceptor cells and inhibitory effect of digestive enzymes (de Oliveira et al. 2019).

After the rosmarinic acid, which is described above, the Rosemary also contains a quite high amount of ursolic acid, which is one of the most used substances in cancer treatment and, at the same time, it has remarkable anti-diabetic properties (Hernández et al. 2016; Hussain et al. 2017).

Thanks to other substances attained from the plant, Rosemary is likewise used for rheumatism, Alzheimer's disease, or to prevent colds (de Oliveira et al. 2019; Aitfella Lahlou et al. 2021).

Figure 4: *Rosmarinus officinalis* L.

Source: Hernández et al. (2016)

4.4. Enhancing the plant’s potential

As mentioned, medicinal plants have a vast number of properties, and their use is extended into many curative areas. However, with the rising demand for healing in traditional ways and because of the worldwide increasing population, there has been developed different ways to increase the plant’s potential and produce an even higher number of secondary metabolites (Iannicelli et al. 2020).

There are many ways, from the crop’s layout on the field, fertilisers, symbiotic organisms, and since the genetic discovery has been a reachable tool, even the plant breeding can be manipulated.

Nowadays, plant breeding takes the front place in the metabolite increasing process. Breading could also provide in parallel higher endurance against any kind of stress and may produce higher biomass. It includes techniques such as crossbreeding, selective breeding, mutation breeding, and also genome doubling, known as polyploidization (Wang et al. 2020; Bhardwaj et al. 2020).

4.5. Polyploidization

It is known about polyploidy for more than one hundred years already (Salma et al. 2017). Since that time, people got to know that genome doubling or polyploidy has been perceived and will continue as a significant power in diversification and evolution, and that most of the existing species have experienced it at least once in their development (Levin 1983; Eng & Ho 2019; Fox et al. 2020). It is a crucial component of surviving and organismal variety, especially in plants (Kyriakidou et al. 2018).

Polyploidization, as a general term Whole-genome duplication shows, collects organisms containing more than two genomes, this kind of hybridization cause changes in the genetic construction and the phenotype of an organism (Fox et al. 2020).

Changes in the genetic information in the cell causes futher morphological and physiological changes, these changes are unpredictable and depends only on nature (Niazian & Nalousi 2020). The first change starts with an increase in the cell's size; the tetraploid cell volume is usually about two times bigger than the diploid one due to the multiplication of the genetic information (Panawala 2017). More effects follow as the enlargement of leaves, flowers, fruits, and roots are also within the changes (Iannicelli et al. 2020). The number and quantity of secondary metabolites are also influenced by these changes; therefore, plants are considered to be more resistant to their environment, and for humans, they have more healing substances; thus, they are having larger economic importance (Salma et al. 2017).

The natural process of polyploidization occurs in a long period of time where the plant is exposed to stress; it could be a wound, a viral infection or ionizing radiation, which activates the generation of a new cell. Consequently, it produces a malfunction during the new cell generation with the addition of a new set of chromosomes to the existing ones. Due to the number of chromosomes sets contained in the cell nucleus, the organism is named diploid, triploid, tetraploid, and it goes on with higher numbers (Fox et al. 2020).

Whereas the polyploidy cell division has two different paths, mitotic, commonly known as somatic, and meiotic doubling (Stuessy & Weiss-Schneeweiss 2019). Related to these divisions, the polyploidy has another two categories that differ from each other by a number of species involved in the chromosome doubling, alloploidy and autoploidy (Dhawan & Lavania 1996).

4.5.1. Mitotic polyploidy

The somatic polyploidization occurs by collapse during chromosome separation within one plant (Stuessy & Weiss-Schneeweiss 2019); for this kind of polyploidy, antimitotic agents are used, which affect the separations mostly during the metaphase, where it disturbs the association of tubulin proteins needed for the formation of the dividing spindle. When a cell comes into metaphase, it contains doubled genetic information, which cannot be divided with the absence of a dividing spindle (Dhooghe et al. 2011). Antimitotic agents are used in controlled conditions for inducing mitotic polyploidy.

4.5.1.1. Antimitotic agents

Antimitotic agents are chemical substances used as alternative inhibitors of dividing spindle. During metaphase, are antimitotic agents bind to the tubulins and stop their growth; tubulins cannot join to the dividing spindle and attach to chromosomes, and in metaphase is causes an alteration after polyploid is formed, the dividing process completely stops (Dhooghe et al. 2011; Ebrahimzadeh et al. 2018; Eng & Ho 2019).

From all antimitotic agents, the most used are colchicine, oryzalin and trifluralin, depicted on Figure 5. Their efficiency depends on concentration, exposure time or application method (Dhooghe et al. 2011; Niazian & Nalousi 2020).

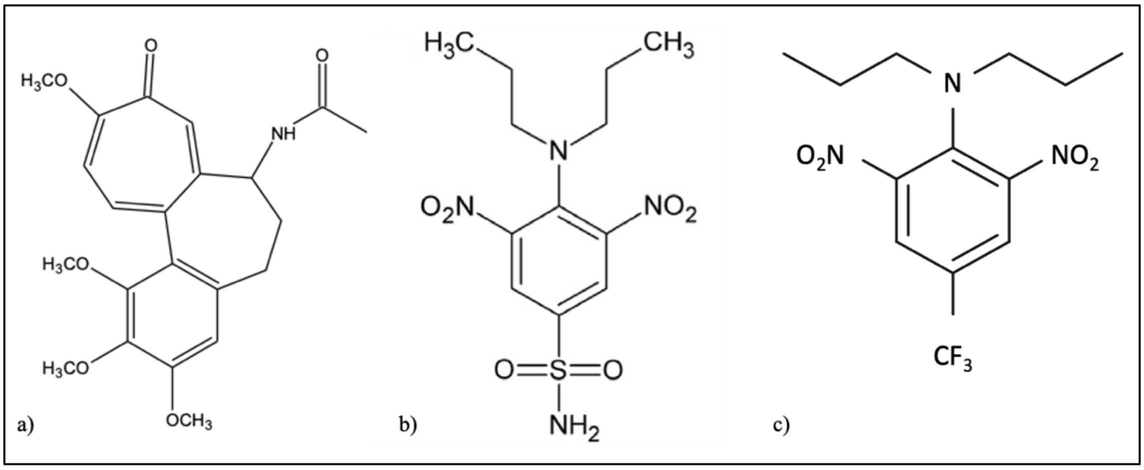

Figure 5: Chemical structure of antimitotic agents

a - colchicine, b - oryzalin, c – trifluralin

Source: Epp et al. (2018); Khatun & Riyazuddeen (2018); Gerent & Spinelli (2019)

Colchicine is the most used for acquisition polyploidy in plants (Niazian & Nalousi 2020), it is a natural alkaloid obtained from *Colchicum autumnale* L., an extremely toxic agent, with only 0.8 mg per kg could be lethal for human, and since it even affects the human cells, being cautious is the most important rule during working with colchicine (Eng & Ho 2019). It is heat and light-sensitive; high temperature or ultraviolet light could aggravate its bioactivity.

Colchicine is affecting the epidermal and subepidermal layer or even more depending on its dosage and application time. The dosage depends on the thickness of the plant cell that it needs to penetrate, but commonly it is from 1.25 to 2.5 mM of solution. The exposure time was reported between 20 minutes and 37 days, but the most common is usually in a matter of hours. These parameters, dosage and time, are very variable and has one common coefficient, the used method (Dhooghe et al. 2011; Gantait & Mukherjee 2021).

Oryzalin and trifluralin are dinitroaniline herbicides. Unlike colchicine, they have a high affinity in tiding up with plant tubulin, where colchicine-binding affinity is higher to mammal tubulin. Therefore, they can be more effective for producing polyploids and safer for human manipulation. They need a larger dosage than colchicine from 1 to 50 mM, and they are less demanding on their storage (Dhooghe et al. 2011; Ebrahimzadeh et al. 2018; Gantait & Mukherjee 2021).

4.5.2. Meiotic polyploidy

The meiotic division is a mightily controlled procedure that provides fertility to all living organisms; hence, slight changes in the process can cause the meiotic polyploidisation to not be often competent to sexually reproduce (Soltis & Soltis 2012). The usual process starts by replicating the genetic information, continued by the division of parental chromosomes, and then dividing the sister chromatids, which form four cells. Through this process, the crossing over applies, which gives variability to the new plant cells by trading part or parts of ales homologous chromosomes (Pelé et al. 2018).

The polyploidization throughout meiosis occurs due to a failure by unreduced gametes; the process of breeding could be reduced by one generation thus is faster than the regular meiosis (Ramsey & Schemske 1998; Iannicelli et al. 2020).

4.5.3. Autoploidy and alloploidy

Autoploidy is a mutation of the same genome of chromosome complement, and it consists of more than two copies of homologous chromosomes (Van de Peer et al. 2017; Pelé et al. 2018).

This kind of ploidy increases the nuclear ploidy, often affecting the physiology and biomass as leaf size, the density of stomata, cell size or number of chloroplasts per cell and other anatomical or structural changes. These changes can distress the plant's biochemical activities, such as its net photosynthesis, transpiration, and enzyme activity, leading to higher biomass (Dhawan & Lavania 1996; Panawala 2017).

During alloploidy replicate two different genomes obtained from various species are combined; the result is a hybrid of two different organisms (Iannicelli et al. 2020). The hybrid is usually infertile between both parental species, and it has significant biochemical and physiological properties combined between both parental organisms (Dhawan & Lavania 1996; Eng & Ho 2019).

4.5.4. *In vitro* polyploidization

In vitro cultivation was first used in 1898; since then, people started to know its advantages compared to the open environment. Unlike in open spaces, plants grown *in vitro* are protected from surrounding factors like bacteria, fungi, viruses, temperature or other effects. The meaning is to grow healthy plants; thus, it is possible to observe effects on substances or stress caused by our manipulation and then have better and more efficient results of experiments (Martin 1985; Salma et al. 2017).

Many years have passed since the first *in vitro* cultivation and many scientists got to know its qualities. In 1966 was reported first *in vitro* polyploidization on tobacco by Murashige and Skoog (Salma et al. 2017). Nowadays the *in vitro* induction of polyploidy is the most used technique of polyploidization (Niazian & Nalousi 2020). It could be for its properties, leading to faster and more controllable inductions or larger variability of explant types. Among the explant types are embryos, seeds, germinated seeds, cluster buds, nodal segments or calluses.

There is much variability in producing polyploids; all types of explants could be cultivated in different medium, treated with a different method and different antimitotic agent, and then determined in different methods (Dhooghe et al. 2011).

4.5.5. Detection of polyploidy

Following polyploidy induction, the experiment reaches the detection part to determine if a relevant change in the genetic information of the plant has occurred. There are few detection methods, from the traditional chromosome counting or morphological observations to complete devices that perform a deep data collection with high accuracy margins. These processes could be separated into two main parts as direct and non-direct methods of detection (Dhooghe et al. 2011).

4.5.5.1. Non-direct methods of detection

Morphological and anatomical changes are among the expected changes in polyploid plants; it is no wonder why these changes are helping with ploidy detection. Roots, leaves, stamps, or flower magnification are the first visible changes, they are detectable easily, only with the need of the human eye and measuring them is still part of the polyploid experiments. As mentioned above, the size is not the only modification; the shape variation is also usual.

Procedures such as the cytological observation of stomata, pollens, and chloroplasts in guard cells observed under the microscope can also be added to the non-direct methods (Pei et al. 2019).

4.5.5.2. Direct methods of detection

While speaking about direct methods, it is considered to get the precise results obtained from recognizing genetic information inside the organism.

Chromosome counting is the most accurate method, seeking to find an exact number of chromosomes (Dhooghe et al. 2011). For chromosome counting, shown on Figure 6, it is usually used 2 cm long root tips, and the counting itself takes place during the metaphase of mitotic division; however, this is a complicated and laborious method.

Besides this procedure, there are also more comfortable and faster detection processes using chromosomes' colouration called the squash technique (Ochatt et al. 2011).

Another widespread determination is flow cytometry used with comparison suspected polyploid organism with control. In this method, the number of chromosomes is not decisive, but the concentration is compared to control (Pei et al. 2019). During this method, plant material is comminuted for release of cell nuclei, then, by measuring the specific optical and fluorescence in the cells is determined the amount of genetical information in a specific plant (Ochatt et al. 2011; Adan et al. 2017).

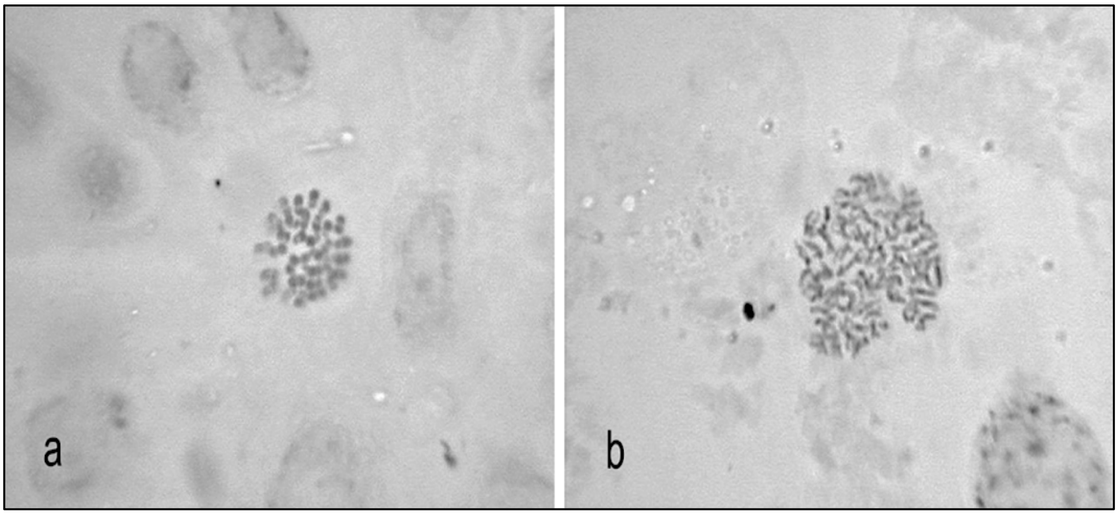

Figure 6: Chromosome counting in *Smallanthus sonchifolius* (Poeppig & Endlicher)

a - octoploid, b - hexadecaploid

Source: Viehmannová et al. (2009)

4.6. Results and discussion

4.6.1. Induced polyploidy in medicinal species in Lamiaceae family

Several kinds of research have been done about polyploidy induction. Even though they hold many differences, they all share the procedure as a common factor depicted on Figure 7.

When inducing the polyploid *in vitro* or *ex vitro* in the beginning, there is plant material. Before induction is the plant material treated and prepared for the addition of an antimitotic agent. The plant material is then exposed to an antimitotic agent in several amounts of concentration for various time period generating impact in the plant tissue. After the exposure occurs, the plant treatment, cultivation, and morphological observation lead to ploidy determination, usually with a direct method. These differences make unique experiments.

Seventeen different plants from nineteen experiments of induced polyploidization were compared (see Table 1); all of them belonged to the Lamiaceae family, and each one of them had a medicinal use. There are two possible ways of evaluating this review, the first consist of revealing the most used way of polyploidy induction, another one lies in determining the most effective induction path.

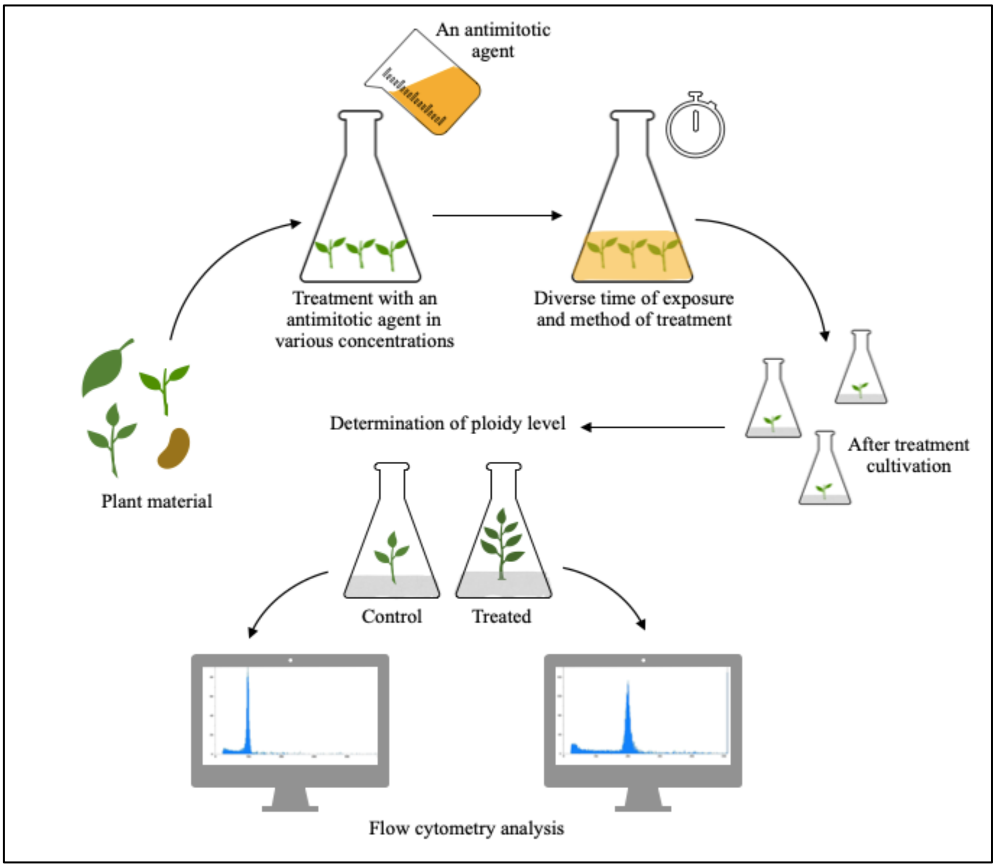

Figure 7: *In vitro* polyploidy induction procedure

Source: Author (2021)

4.6.2. Effectivity of used methods

Every single plant reacts differently depending on the treatment. As mentioned, the most significant variables on polyploidization are the parts of the plant, treatment methods, antimitotic agents and duration of exposure. The choice of parameters is done by research of plants with similar characteristics and their selection, after these factors are appropriately combined a successful polyploidy can be expected (Salma et al. 2017).

In the following chapters, plant parts, treatment methods, antimitotic agents and exposure duration from all the investigated experiments, were compared according to their efficiency in the percentage of total achieved polyploid plants. These percentages were obtained either directly from a relevant article or calculated as the average of the total number of plants, treated by the most efficient method; and from the final number of polyploids. The more explants are exanimated, the more precise are the efficiency percentages.

4.6.2.1. Use of plant parts

Among the studied species the most common plant parts used for induction were the nodal segments (see Figure 8) and seeds at emergence stage, followed by seeds. These two seed explant types made the most usual group from all other plant parts with 48 % of use, including experiments such as *Lavandula angustifolia* L. (Jordanov et al. 1995; Urwin et al. 2007) or *Tetradenia riparia* Benth. (Hannweg et al. 2016b).

Considering that the use of plant part is compared with the number of total efficiencies, the cluster bud from the *Pogostemon cablin* Benth. (Yan et al. 2016) would take the first place in scale. Cluster buds were used twice in this research, besides *Pogostemon cablin*, it was also used in *Salvia miltiorrhiza* Bunge (Gao et al. 1996) and their average efficiency was 74 %. Nevertheless, this explant has been used in small quantities; therefore, this efficiency must be taken with caution.

Figure 8: Use of nodal segment

Source: Author (2021)

Another plant part with a high representation was the nodal segment, the published experiments that used this method did not include any other plant part for induction; thus, the plant effectivity was not compared between other methods. The average efficiency using the nodal segment was 26 %, making this explant the second most efficient from investigated plants. The success of the nodal segments in polyploidy induction was recorded in numerous experiments with plants from different families (see Table 1). In the past year, several articles were published with use of nodal segments including plants as *Chrysanthemum* cv. Gongju Ramat. (Yue et al. 2020), *Chrysanthemum boreale* Makino (Hoang et al. 2020) or *Physalis alkekengi* L. (Santos et al. 2020).

The diversity of the chosen plant parts is usually very high, but their simplicity and efficiency differ from one another. Salma et al. (2017) have reported that seeds are the most appropriate explant for polyploidy induction since the seed cells are in an active stage of division, therefore the antimitotic agent can penetrate them easier. The only operation before the induction is sterilising them, aiming to make them easy to manipulate.

4.6.2.2. Use of an antimitotic agent

As mentioned before, the most used antimitotic agent is colchicine, these can be seen in the reviewed experiments, where this agent was predominant over the others. After the colchicine the oryzalin follows, being used in two experiments; finally, the trifluralin was used merely once.

A higher mortality of plants occur with higher volume or concentration of antimitotic agent, and a lower concentration may not give the right impulse to derive in polyploid plants (Dhooghe et al. 2011; Niazian & Nalousi 2020). In the reviewed experiments, a high concentration or an extended time period to the agent were often deadly to the plant. This phenomenon was seen in multiple reviewed articles as in the induction of *Salvia leriifolia* Benth. (Estaji et al. 2017), *Dracocephalum kotschyi* Boiss (Zahedi et al. 2014) or S*cutellaria baicalensis* Georgi (Gao et al. 2002). The higher survival rate usually responds to an adequate concentration with an elevated number of polyploid plants.

The exposure time was from 0.5 to 30 days, simultaneously, the concentrations levels decreased with time, describing a direct relationship with the exposure time. The higher quantity of polyploids was observed after 72 hours of exposure; this time was the second most efficient treatment time. The most used exposure time was 12 hours, together with an overnight treatment, and this time of duration also had the highest percentage of created polyploids. As is visible in Table 1 the duration of 24 hours had closest relation within time and an average amount of colchicine from 0.2 to 0.5 % (w/v) (Grouh et al. 2011; Hannweg et al. 2016a; Ehsani et al. 2017; Hassanzadeh et al. 2020).

4.6.2.3. Treatment method

The treatment methods in this research were performed in two different places, *in vitro* and *ex vitro*. *In vitro* cultures' effectivity should not be denied, as noted by Dhooghe et al. (2011) and published in other experiments and reviews (Esfahani et al. 2020; Zhou et al. 2020; Gantait & Mukherjee 2021). Despite that the *in vitro* method is quite efficient, the results must be treated and measured carefully, controlling that the polyploids are not under the "*in vitro* effect" instead of the "polyploid effect" as mentioned by Iannicelli et al. (2020).

The observed plants confirm the hypothesis about the effectivity of *in vitro* induction effectivity; not only the plants that grew under *in vitro* conditions, had an average effectivity 17 %, but also this condition was used more often than *ex vitro*.

Besides the place of treatment, it is important the method itself. It answers the questions: how the explant absorbs the antimitotic agent, and in which condition. The antimitotic agents are used in many ways (Iannicelli et al. 2020); the simplest would likely be the soaking method, used besides the observed plants, in *Citrus limon* L. (Bhuvaneswari et al. 2020) or *Zingiber officinale* Roscoe (Zhou et al. 2020).

In this review four treatment methods were used; cotton ball method, liquid or solid medium supplemented with a solution of an antimitotic agent and the plant material soaked in a solution. Probably for its simplicity the soaking method was applied the most, with 58 % of use. For instance, the soaking method was used when treating *Thymus persicus* Ronniger ex Rech. f. (Tavan et al. 2015) or *Thymus vulgaris* L. (Homaidan Shmeit et al. 2020). This method was followed by inductive medium (32 %) with or without an addition of agar, as in the case of *Pogostemon cablin* Benth. (Yan et al. 2016).

A percentage average of effectivity was done from the plants which used the same method. The most effective method appeared to be the inductive medium to be seen in the Figure 9, represented in both states. This method attained 27.71 %. On the other hand, the lowest efficiency was described by the soaking method with a 13.36 %

of effectivity, and the cotton balls stood in the middle, represented only by two plants (Zahedi et al. 2014; Estaji et al. 2017).

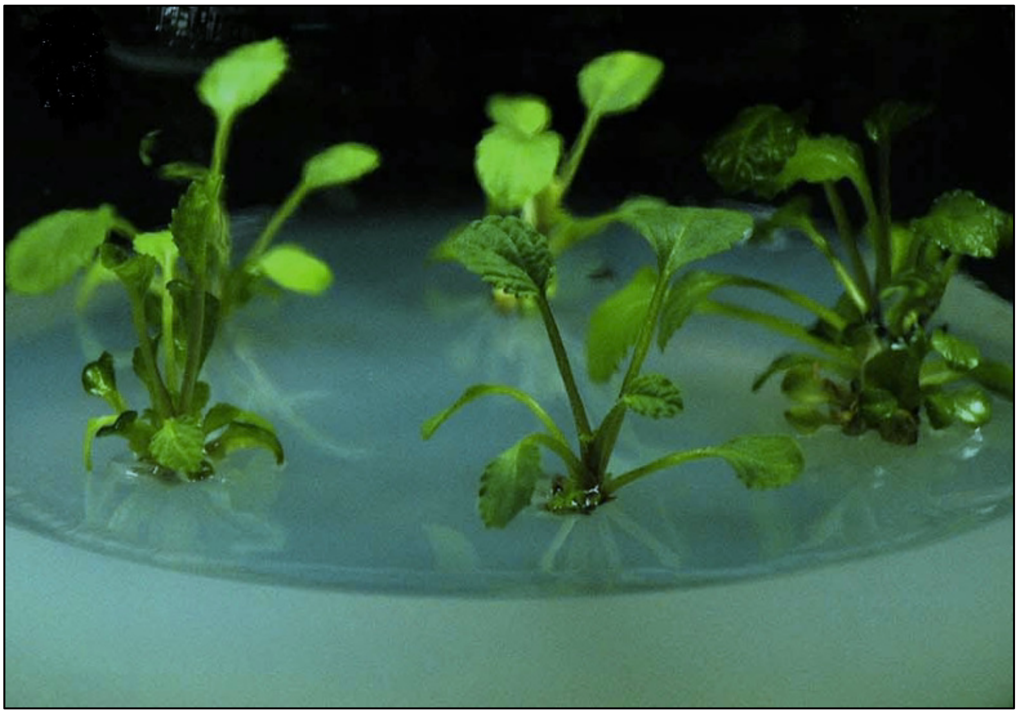

Figure 9: *In vitro* - inductive medium method preformed on *Salvia miltiorrhiza* Bunge

Source: Chen et al. (2018)

4.6.3. Morphological changes in polyploid plants

The increase in the volume of plant organs is due to gigantism because there is a close relationship between the chromosome number and nucleus size, as well as between the nucleus and the cell size, so polyploid gigantism is due to larger cell size (Sliwinska 2018).

The reviewed experiments recorded various morphological changes, and most of them were registered in *Thymus persicus* (Ronniger ex Rech. f.) (Tavan et al. 2015). Notified modifications of the observed species were change of height; stomata number, length and frequency; changes of leaf size, number or shape; change of thickness of various plant parts; length of root and other physical attributes.

There were many common differences within polyploid plants. The most common morphology decrease was the stomata density which was accompanied by an increased stomata size as shown in Figure 10. This stomata difference was recognised in 12 plants. Other decreases were recorded in the plants' height number of branches, shorter leaves, shorter root or shorter height.

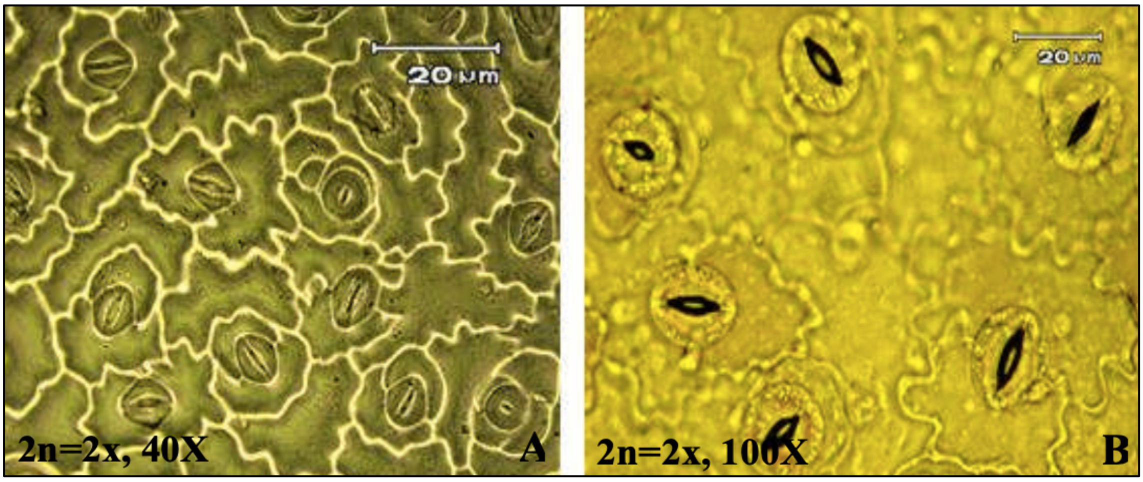

Figure 10: Comparison of stomata density on *Dracocephalum moldavica* L.

A – diploid; B – tetraploid plant

Source: Omidbaigi et al. (2010)

On the other hand, the number of species with increased morphological characteristics were more abundant. The most frequent increases were larger seeds and pollen, growth of the fresh or dry mass, darker colour, and most of the seen was the extension of the leaf or stem thickness observed in 11 experiments including *Monarda fistulosa* L. and *Monarda punctata* L. (Moeller 2015), *Ocimum basilicum* L. (Omidbaigi et al. 2012) or *Thymus vulgaris* L. (see Figure 11).

Figure 11: Morphological differences on *Thymus vulgaris* L.

A – diploid, B – tetraploid

Source: Homaidan Shmeit et al. (2020)

4.6.4. Biochemical changes and their impact on medicinal uses

An expansion of the size of various plant parts increases the biosynthesis of enzymes, leading to quantitative and qualitative changes of secondary metabolites. These changes could have a medicinal value, and that is why there are many articles devoted to this subject, as Salma et al. (2017), Niazian & Nalousi (2020) or Iannicelli et al (2020).

In most of the investigated plants, differences were found in the increase of the total amount of essential oils; various compounds experienced an increase in their amount, or even completely new compounds were found in the polyploids, absent in the previous control plants. The tetraploid of *Dracocephalum moldavica* presented an increase of total content of essential oils by 27.5 % (Omidbaigi et al. 2010).

Other plants as *Dracocephalum kotschyi* Boiss, *Melissa officinalis* L., *Monarda punctata* L. and *Salvia officinalis* L. reported a complete increase of phenolic or flavonoid content (Zahedi et al. 2014; Moeller 2015; Talei & Fotokian 2020; Hassanzadeh et al. 2020).

Besides the growths of the biochemical components in the plants, there was also a reduction in numerous compounds or their complete nonexistence.

Each plant among the studied species holds various medicinal properties, and after the set of chromosomes duplication multiple changes can occur in their healing effects. Some species did not include chemical analysis, or their results were not relevant; hence they are displayed only in Table 1. The reviewed species with various differences were *Dracocephalum kotschyi* L., *Dracocephalum moldavica* L., *Lavandula vera* D.C., *Monarda fistulosa* L., *Monarda punctata* L., *Salvia leriifolia* Benth., *Salvia miltiorrhiza* Bunge, *Tetradenia riparia* Benth., *Thymus persicus* (Ronniger ex Rech. f.) and *Thymus vulgaris* L.

4.6.4.1. ***Dracocephalum kotschyi*** **L.**

Dracocephalum kotschyi is traditionally used for many health disorders, a change of amount in secondary metabolites occurred by polyploidy induction. The most remarkable change was visible between the diploid and tetraploid concentration in calycopterin, xanthomicrol and penduletin. The increase of these compounds can also increase the pharmacological effects; all of these three compounds are known for their antitumor propreties (Heydari et al. 2019). The calycopterin can increase the immunoinhibitory effects of a tetraploid plant (Faham et al. 2008; Esmaeili et al. 2014) and the xanthomicrol is more known for its anti-inflammatory effects (Guo et al. 2018a; Ghazizadeh et al. 2020).

There was a slight decrease of cosmosiin, apigenin, cirsimaritin and isokaempferide, therefore it could be assumed that the antioxidant or anticancer properties did not decrease greatly.

4.6.4.2. ***Dracocephalum moldavica*** **L.**

The content of geraniol and Z-citrial increased in polyploid plants of *Dracocephalum moldavica* (Omidbaigi et al. 2010), but other measured components decreased. The enlargement of Z-citrial can derive into an improvement of the plant's antioxidant potential along with the geraniol, which also contain antimicrobial effects (Ehsani et al. 2017; Lira et al. 2020).

A minor loss of nerol, E-citral, neryl acetate and a high decrease of geranyl acetate contents occurred.

4.6.4.3. *Lavandula angustifolia* Mill.

The *Lavandula angustifolia* or *Lavandula vera* was represented in this review by two articles. The Lavandula, published by Urwin at al. (2007) not mentioned chemical research, there was only a comparison between the growth of the flower diameter and the possible increase of essential oils.

On the other hand, autoploidy induction of *Lavandula vera* performed by Jordanov et al. (1995) mentioned a total increase of essential oil content. The article mentioned yield and reduction of several biochemical compounds: mircene, b - ocimene and limonene, cineole, linalool, lynalylacetate, lavandulol and terpinene1-ol-4. In all promising clones were different amounts of components, but their increase would improve anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antiviral, gastroprotective effects, extrinsic amelioration of skin aging or other benefits (Hwang et al. 2017; Vieira et al. 2018; Kim et al. 2019).

4.6.4.4. *Monarda fistulosa* L. and Monarda *punctata* L.

The *Monarda fistulosa* polyploidization (Moeller 2015) displayed a significant decrease of antioxidant activity compounds by contrast with the control diploid plant. It also lowered the percentage of phenolic compounds.

Despite the decrease of healing goods of *Monarda fistulosa, Monarda punctata* (Moeller 2015) somewhat improved the antioxidant activity compounds together with a notable increase of phenolic compounds.

4.6.4.5. *Salvia leriifolia* Benth.

The extract of essential oils of *Salvia leriifolia* (Estaji et al. 2017) exhibited an increase of total percentage of essential oils and also eight new compounds which were not found in diploid plants; α-copaene, aromadendrene, γ-muurolene, cis-β-guaiene, trans-β-guaiene, α-eudosmol, δ-cadinene, α-cadinene. The compound with highest presence in tetraploid was 1,8-cineole. Together with other increased compounds these changes can lead to a higher antimicrobial, anti-inflammatory or antitumor activities (Mulyaningsih et al. 2010; Rufino et al. 2015; Casiglia et al. 2017).

The number of camphene and Δ-cadinene fallen to zero in tetraploids and the other eight components decreased in correlation with a higher chromosome number.

4.6.4.6. *Salvia miltiorrhiza* Bunge

Polyploidy of *S. miltiorrhiza* was performed twice, and both publications shared the chemical analysis results obtained from the root of an autotetraploid (Gao et al. 1996; Chen et al. 2018).

The article from the year 1996 mentioned that the best plant from the experiment increased the total productivity of tanshinones (I and II), and cryptotanshinone (Gao et al. 1996).

The second article published by Chen et al. (2018) described the same phenomenon on the total amount of increased tanshinones, but there were no other significant changes in the single components, except for a higher amount of dihydrotanshinone.

Tanshinones are carrying anti-Alzheimer, anticancer or atheroprotective effects; thus, the use of plants with a higher amount of these components would provide a high profile effectivity in multiple medicinal areas (Fang et al. 2018; Fu et al. 2020).

4.6.4.7. *Tetradenia riparia* Benth.

There was a reported significant increase of some essential oils as fenchone, the appearance of three other compounds which were not found in diploid plants was also disclosed: a-humulene, a-terpineol, viridiflorol in *Tetradenia riparia* (Hannweg et al. 2016b). The growth of fenchone may result in competent agents against diarrhoea or wound healing (Keskin et al. 2017; Pessoa et al. 2020).

The study results demonstrated a large decrease of fenchyl acetate and isopimara-8,15-diene, these two compounds were the major components of diploid *Tetradenia riparia*. Their reduction can lower the anti-inflammatory effects of the plant (Nwet Win et al. 2020).

4.6.4.8. *Thymus persicus* (Ronniger ex Rech. f.)

The induction of the tetraploid plant caused a significant rise of betulinic, oleanolic and ursolic acids (Tavan et al. 2015). The ursolic acid that grows over 140 % is used in pharmacology for anti-inflammatory and antihyperlipidemic purposes and against several chronical diseases involving cancer, brain disease, and others (Jie 1995; Seo et al. 2018).

Betulinic and oleanolic acids are having the same properties as the ursolic acid but also antiviral, antidiabetic, anti-inflammatory have been recorded; it also can be used in smaller dosages as a hepatoprotective agent (Ríos & Máñez 2018; Liu et al. 2019).

4.6.4.9. *Thymus vulgaris* L.

The *Thymus vulgaris* (Homaidan Shmeit et al. 2020) tetraploids elevated the amount of thymol and carvacrol production. As mentioned, thymol is used as an antioxidant and anti-inflammatory agent to treat digestive, cardiovascular or nervous system disorders. Carvacrol is also demanded for its antimicrobial activity (Salehi et al. 2018; Sharifi-Rad et al. 2018).

However, a significant decrease was documented in components used for antinociceptive, antimicrobial and anti-inflammatory functions (de Souza Siqueira Quintans et al. 2013). These components are p -cymene and γ-terpinene, which together with thymol represent major diploid plant compounds. A large reduce would possibly decrease the pharmacological activity of these compounds.

Table 1: Summarised table of induced polyploids in Lamiaceae family

Plant spp.	Treated plant part; treatment method	Antimitotic agent, its concentration, and exposure duration	Resultant ploidy level	Polyploidy efficiency; number of tested plants	Morphological changes of polyploid plants	Chemical changes of polyploids	Medicinal use*	References
Dracocephalum kotschyi Boiss	shoot apical meristem; cotton ball method	Colchicine, 0.5 % (w/v), 48 hours	Diploid to Tetraploid (2n = 4x = 40)	12 %; 25	Increase of stomatal lengths, widths and the steam diameter; Decrease plant height, leaf number, and lateral branches number.	The total increase of flavonoids about 306 (µg/g DW) more in stable tetraploid; concentration of four compounds decreased	Antirheumatic, antitumor, antimutagens, antioxidant, antiseptic, analgesic, antispasmodic, has stimulant properties, antidiarrheal activities, and treating gastrointestinal disorders	(Zahedi et al. 2014)
Dracocephalum moldavica L.	seeds at the emergence of two true leaves; soaked in solution	Colchicine, 0.1 % (w/v), 72 hours	Diploid to Tetraploid (2n = 4x = 20)	7.2 % -	Increased size of seeds higher diameter and higher length of stomata and stomatal guard cells, in leaf area, the fresh and dry mass of plants, and the size of seeds, increase of leaf area; Decrease plant height.	Up to 27.5 % increase of essential oil content	Antioxidant activity, antiseptic, analgesic, anti-inflammatory, antibacterial and is anticonvulsive and sedative	(Omidbaigi et al. 2010)
Lavandula angustifolia Mill.	seed; soaked in solution	Colchicine, 125 and 15.6 mg/l, week	Diploid to Tetraploid (2n = 4x = 90+)	0.5 % 400	Larger flowers, leaves, and trichomes; thicker peduncles and thicker leaves; The number of whorls of flowers per spike did not change	-	Antirheumatic, antiseptic, analgesic, antimicrobial, emmenagogue, it is an expectorant, tranquillizer, and it is used against digestive problems and as postpartum care	(Urwin et al. 2007)
Lavandula vera D.C.	seed; soaked in solution	Colchicine, 0.01 % (w/v), till germination	Diploid to Tetraploid	11.4 % 366	2 to 2.2 times bigger, pollen enlarged.	The total content of essential oils in some clones enlarged about more than 1 %		(Jordanov et al. 1995)
Melissa officinalis L.	germinated seed; soaked in solution	Colchicine, 0.2 % (w/v), 24 hours	Diploid to Tetraploid (2n = 4x = 64)	-	Increase of stomata length, stomata width, and dry weight. Decrease stomata density.	A slight decrease in the rosmarinic acid content, and increase of flavonoid content, phenolic acids, and carotenoid.	Antioxidant, anti-inflammatory, anticancer, antimicrobial and antidepressant, it can treat sleep disorders, neurodegenerative diseases, obesity, and digestive problems	(Talei & Fotokian 2020)
Monarda fistulosa L.	nodal segments; inductive media	Oryzalin/Triflurali, 15µM / 60µM, 72 hours	Diploid to Tetraploid (2n = 4x = 72)	21 %; 89	Increased leaf size, darker and thicker leaves.	A significant decrease in antioxidant activity of the tetraploid extracts and lowered the phenolic content	Antibacterial, antiviral, anthelmintic, antioxidant, analgesic, expectorant, immunomodulatory, radioprotective, diuretic carminative and anti-inflammatory, anti-tumor, treatment of digestive disorders.	(Moeller 2015)

Monarda punctata L.	nodal segments; inductive media	Trifluralin, 60µM, 72 hours	Diploid to Tetraploid (2n = 4x = 44)	9.6 %; 62	Increased leaf size, darker and thicker leaves.	An increase in the phenolic content observed in the tetraploid and a small increase of antioxidant activity compounds	Anti-inflammatory, anthelmintic, carminative, diuretic, expectorant, treatment of digestive disorders.	(Moeller 2015)
Ocimum basilicum L.	seedlings at the emergence; soaked in solution	Colchicine, 0.5 % (w/v), 72 hours	Diploid to Tetraploid (2n = 2x = 96)	8 %; 100	Enlarge of stomata and pollen grains, chloroplast number in guard cells. Leaves were thicker, dark green, more dissected and dentated at their margins, had a larger seeds and pollen grain; Decrease stomata density.	-	Nematocidal, antibacterial, antifungal agents and antioxidant, helps with kidney malfunction.	(Omidbaigi et al. 2012)
Plectranthus esculentus N.E.Br.	nodal segments, soaked in solution	Colchicine, 1.0 g/l, overnight or 72 hours	Diploid to Tetraploid	26.8 %; 250	Larger darker shade of green leaves than the diploids; Thicker stems; No difference in plant height fewer stems.	Decrease of amount ash, protein, carbohydrates, alanine, aspartic acid; markable increase amount of fat, starch, beta-Carotene, histidine; Some elements changed their content	Digestive, analgesic, anthelmintics, abdominal pain, cytotoxic and anti-tumor.	(Hannweg et al. 2016)
Pogostemon cablin Benth.	Cluster buds (3–6 mm); inductive liquid media	Colchicine, 0.05 % (w/v), 72 hours	Tetraploid to Octoploid (2n = 8x = 128)	68 %; 40	Taller and more robust with larger leaves and stomata; thicker stems.	Increase patchoulic alcohol content for even more than 3 mg/l, only two lines, had a lower content of patchoulic alcohol than the control.	Antimutagenic, antithrombotic, antiemetic, antioxidant, antimicrobial, cytotoxic, improve digestion and relieve heat.	(Yan et al. 2016)
Salvia hains Royle ex Benth.	germinated seed; soaked in solution	Colchicine, 0.5 % (w/v), 24 hours	Diploid to Tetraploid (2n = 4x = 32)	5.2 %; 250	Larger stomata length, stomata width, chloroplast number in a granular cell, an increase of the leaf's length, the width of leaf, leaf index, an internodal distance of stem, fresh mass, and dry mass; Low stomata frequency, plant height, length and number of branches.	-	Analgesic; treating against arthritis, anxiety, eczema and body swelling.	(Grouh et al. 2012)
Salvia leriifolia Benth.	shoot apical meristems of seedings; cotton ball method	Colchicine, 0.05 % (w/v), 48 hours	Diploid to Tetraploid (2n = 4x = 44)	23.3 %; 30	Larger stomata length, stomata width, guard cell length; significant increase in the number and thickness of leaves, leaf width, surface, shoot fresh and dry weight, root fresh and dry weight of tetraploid plants, higher total chlorophyll content of leaves; Low stomata frequency, decrease in plant height and leaf length.	Eight new essential oil compounds were found in tetraploid plants. Two compounds were recognized in diploid plants, which were not observed in tetraploids. Other compounds were in tetraploid in a lower amount than in diploid. Total content enlarged about 3 %.	Antinociceptive, anti-inflammatory, anti-ischemic, anticonvulsant, antiulcer, anti-mutagenic effects and antibacterial, sedative, antihyperglycemic, skeletal muscle relaxant, analgesic, neuroprotective.	(Estaji et al. 2017)

Salvia miltiorrhiza Bunge	Cluster buds; inductive media	Colchicine, 10 ppm, 30 days	Diploid to Tetraploid (2n = 4x = 48)	12 %; 100	Leaves were thicker, rougher and larger than diploid plants; bigger stomata; Larger stems and roots and longer with dark color; higher average fresh weight of the root; significantly; Fewer stomata.	Six tetraploid lines showed much higher tanshinone productivity than the control; total percentage of tanshinones and cryptotanshinone in tetraploids was mostly lower than in diploids.	Cardiovascular protection, antioxidative, neuroprotective, antifibrotic, anti-inflammatory, and anticancer, analgesic.	(Gao et al. 1996)
	Leaf segments; inductive media	Colchicine; 0.05 mg/l; 3 weeks	Diploid to Tetraploid;	39 %	Larger stomata length and width, larger fresh weight, shoot length, root diameter, number of leaves bigger pollen and seed; Shorter root length, Low stomata frequency,	The total amount of tanshinones increased; No significant difference in the content of salvianolic acid B but higher amount of dihydrotanshinone, other changes were not markable.		(Chen et al. 2018)
Salvia officinalis L.	seeds; soaked in solution	Colchicine, 0.25 and 0.5 % (w/v), 24 or 48 hours	Diploid to Tetraploid (2n = 4x = 28)	9.46 %; 900	Larger size of stomata; bigger leaf length, number width; plant height, node number, internode length; Lesser stomata frequency.	An increase amount of Phenol (6.8mg), Flavonoid (2.58mg), but total amount of protein and antioxidant activity decreased.	Antioxidant, anti-diabetic, anti-inflammatory, antimicrobial, anticancer and hypolipidemic, potential cognitive properties.	(Hassanzadeh et al. 2020)
Scutellaria baicalensis Georgi	callus; inductive media	Colchicine, 0.2 % (w/v), 12 hours	Diploid to Tetraploid (2n = 4x = 36)	16.7 %; 30	The stomata on the leaves' surface were large; rougher, larger and thicker leaves; Low stomata frequency.	Most tetraploid plants had a little smaller baicalin content than that in control, but most tetraploid plants showed higher yield of baicalin per plant.	Anti-inflammatory, treatment of diseases as trachoma hepatitis, hypertension, detoxification, and promoting digestion, acute respiratory infection, acute gastroenteritis, infantile diarrhea, vomiting during pregnancy and others.	(Gao et al. 2002)
Tetradenia riparia Benth.	seeds; soaked in solution	Colchicine, 0.01 g/l, overnight	Diploid to Tetraploid	25.6 %; 100	There were no apparent differences in plant height (and no difference in internode length); thicker and stickier leaves; more rounded leaves (diploids were cordiform). Shorter leaves.	Tetraploid plants produced 3.5 times more oil than diploid plants on a fresh weight basis.	Antipyretic, anti-infective - diarrhea, gastroenteritis, stomachache, throat or chest infections.	(Hannweg et al. 2016)

Thymus persicus (Ronniger ex Rech. f.)	top three nodal segments; soaked in solution	Colchicine, 0.3 % (w/v), 12 hours	Diploid to Tetraploid (2n = 4x = 56)	43 %; 60	Length and width of the guard cells were greater; stomata of polyploid plants were larger; Leaves were with significantly more trichome density and a darker green color; The dry and fresh weight of tetraploid plants was higher; No differences were identified in the number of nodes but lesser internode length. Slower rooting growth: decrease number of shoots, shoot length, and root length.	The tetraploid plants showed a significant increase of 69.73 % Betulinic acid, 42.76 % Oleanolic acid and Ursolic acid 140.67 %	Anti-inflammatory, antitumor, antimicrobial, antifungal, anti-HIV, anti-inflammatory, antiulcer, antispasmodic, antihyperlipidemic, hepatoprotective, carminative, digestive, emmenagogic, gastroprotective.	(Tavan et al. 2015)
Thymus vulgaris L.	nodal segments; soaked in solution	Oryzalin, 80 µM, 48 hours	Diploid to Tetraploid (2n = 4x = 60)	7.5 %; 40	There is an increase in main plant thickness, leaf length, leaf breadth, leaf thickness, and plant height in tetraploid plants.	The essential oil content was markedly higher in tetraploid plants. A significant increase in the thymol (18,01 %), p-cymene (10,8 %), γ-terpinene (6,26 %).	Anti-inflammatory, antioxidant and immunomodulatory; effective against various disorders of the respiratory, cardiovascular and nervous systems.	(Homaidan Shmeit et al. 2020)

* Medicinal use before treatment

5. Conclusion

The aim of plant improvement is focused on yield expand, nutritional quality and other characteristics for commercial value and medicinal use. The polyploidy induction forms one of the most important methods for plant enhancement.

This review attempted to explore plants from the Lamiaceae family on which was performed polyploidy induction. On balance, these plants were meant to contain compounds useful in the medical area. The research included nineteen experiments of seventeen plants, finished by attaining duplication of chromosomes. It was found the possibility that the inner change of biochemical compounds may increase the pharmacological and medicinal importance of studied plants rather than their decrease. Thus, the method of polyploidization is henceforward recognized as a method suitable for achieving this goal.

This thesis could be beneficial for the following plant breeding of the Lamiaceae family and provide a more efficient polyploidization process for future experiments.

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