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REVIEW ARTICLE

TISSUE CULTURE TECHNIQUES OF MEDICINAL AND AROMATIC PLANTS: HISTORY, CULTIVATION AND MICROPROPAGATION

Betül AKIN

Kütahya Dumlupinar University, Faculty of Arts and Sciences, Department of Biology, Kütahya, betul.akin@dpu.edu.tr,
ORCID: 0000-0002-2325-7496

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ABSTRACT

Medicinal aromatic plants, which are source of secondary metabolites, have been used for treatment and other purposes since ancient times. In recent years, people have preferred medicinal and aromatic herbs to be healthy. Plant tissue culture methods have the potential to produce medicinal compounds such as secondary metabolites from plants as an alternative to traditional agriculture. Increasing population and increasing demand for herbal products, unconscious collection and illegal trade cause the extinction of medicinal plants in natural habitats. Therefore, it is important to cultivate medicinal and aromatic plants to protect biodiversity and endangered species. As a result, plant tissue culture methods are an alternative way for propagation of medically and economically important plants, the production of bioactive components for the pharmaceutical industry, and the production of medically important secondary metabolites.

Keywords: *Cultivation, Medicinal and aromatic plants, Plant tissue culture, Propagation, Secondary metabolite.*

1. HISTORICAL DEVELOPMENT, DEFINITION AND USE OF MEDICINAL AND AROMATIC PLANTS

Medicinal and aromatic plants undoubtedly have been widely used in many fields such as food, paint, cosmetics, perfume and medicine for centuries. In traditional systems, drugs have been obtained using various plant species grown in different parts of the world. Medicinal plants have been intriguing for humans since ancient times, and the first humans often used aromatic herbs. Medicinal and aromatic plants were frequently used in the prevention and treatment of diseases in traditional medicine during the Neolithic periods, and medicinal plants still continue to be the main types of drugs used in the world. Information about medicinal plants used by humans for therapeutic purposes in ancient times has been transferred to this day and this information is still valid today. Despite the decrease in interest in medicinal plants with the production of synthetic drugs in the 20th century, medicinal products of herbal origin regained importance towards the end of the 20th century [1-6,7].

In ancient times, people were in search of medicine in nature to combat diseases. Since there was not sufficient information about diseases and treatment methods with plants at that time, people learned how a plant could be used in treatment by experience [8,9]. In 3000 B.C., Egyptians used 500 wild

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and cultured plant species in medicine and thus “Phytotherapy”, namely, treatment methods using plants, emerged. However, in 2700 B.C., people in China used cinnamon and the Ancient Greeks referred to the spices as "fragrant aromatic herbs". In the past, methods of obtaining ethereal oil from fragrant plants were developed by humans. In fact, methods of obtaining rose oil were mentioned in ancient sources and in 3500 B.C., people obtained dye from plant roots. More than 600 medicinal plants were examined in a study of 5 volumes titled “De Materia Medica” by Dioskorides, and the use, side effects, dosages and plant growing techniques of drugs were discussed in detail. Some of the drugs that Dioskorides studied are still used today [2,6,10,11]. Medicinal and aromatic plants are generally defined as herbal medicines used as components of natural health products in such areas as pharmacy, cosmetics, nutrition, medicine and perfumery. Medicinal and aromatic plants can also be defined as plants used to prevent diseases, maintain a healthy life and cure diseases [12-15]. The use of herbal medicines is growing rapidly in developed countries and complementary-alternative medicine and herbal medicines are widely used by people with higher education and income levels. With the rapid growth of the population in recent years, people prefer herbal remedies or products to improve their health conditions. Approximately 40% of the compounds used in the pharmaceutical industry are obtained from plants directly or indirectly. Medicinal plants are globally valuable herbal resources, and the majority of these plants are obtained by harvesting from nature and some by cultivation. Therefore, many plant species showing medicinal properties are threatened with extinction due to excessive collection from nature [9, 16-18]. In the last century, as well as the rapid development and increasing side effects of synthetic substances, the advances in knowledge of chemistry and biology, which allow new molecules to be isolated from plants, animals and microorganisms, have led to increased interest in traditional medicines today. With the growing demand from consumers for herbal medicines, natural health products and secondary metabolites, the use of medicinal plants is growing rapidly worldwide. The use of medicinal plants has become important in recent years due to their low side effects [7,18,19,20].

2. CULTIVATION AND PROPAGATION OF MEDICINAL AND AROMATIC PLANTS IN TURKEY AND IN THE WORLD

Located in the temperate zone, Turkey, with the influence of geographical factors, is rich in plant diversity compared to many other countries and the number of plants distribution in Turkey is close to the number of plants distribution in the whole of the European continent. People have used plants for various purposes since ancient times, especially for their health benefits and especially since the 1990s, demand for medicinal and aromatic plants has increased. Many plant types widely used in Anatolia are still used as therapeutic today [10,11,21,22,23]. However, significant parts of medicinal and aromatic plants in the world and in our country are collected from nature, and the rest are cultivated. Although some of the economically important medicinal and aromatic plants used in many fields can be produced easily, there are difficulties in production of some. Thus, only approximately 10% of these plants are cultivated. In Turkey, also the plant cultivation rate is not adequate [14,24]. Accordingly, production area and production amount of medicinal and aromatic plants in Turkey are given by years in Table 1 [25]. According to Table 1 showing the production area and production amount of some medicinal and aromatic plants in Turkey between 2012-2018, opium poppy ranks first among cultivated plants as of 2018 with 26 991 tons of production, followed by thymus with 15 895 tons of production and rose with 14 773 tons of production respectively.

It is stated that 28,187 plant species are used medically in the world; however, only 4478 of the species used in plant-based medicines are shown in the publication as a medicinal regulatory. The

global herbal medicine market is growing rapidly every year. Today, commercial production of some anticancer drugs from herbal sources is carried out successfully [6,26]. Worldwide, the production amounts of medicinal and aromatic plants cultivated for commercial purposes in 2017 and the producer countries are given in the FAO statistics. According to the production amounts of medicinal and aromatic plants worldwide are examined, garlic, ginger, anise, badian, fennel, coriander are among the top places. On the other hand, when the producer countries are evaluated, India and China, which are rich in biodiversity, are at the forefront (Table 2) [27].

Table 1. Production area (decare) and production amounts (ton) of some medicinal and aromatic plants in Turkey by years [25].

Item	**Unit**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Anise	Area (Decare)	194430	152431	140506	138118	136552	121833	124455
	Production (Ton)	11023	10046	9309	9050	9491	8418	8664
Thyme	Area (Decare)	94283	89137	92959	104863	121127	121472	139061
	Production (Ton)	11598	13658	11752	12992	14724	14477	15895
Black Cumin	Area (Decare)	2299	3261	1717	4681	23160	32560	33864
	Production (Ton)	161	352	140	425	2527	3094	3322
Fennel	Area (Decare)	15775	13848	15848	15512	17503	16525	23400
	Production (Ton)	1862	1994	2289	1461	2464	2022	3067
Coriander	Area (Decare)	11	11	11	150	503	410	405
	Production (Ton)	1	1	1	11	42	29	29
Caper	Area (Decare)	-	-	15	15	3	-	-
	Production (Ton)	-	-	-	-	-	-	-
Opium poppy (Capsule)	Area (Decare)	135106	322773	266212	615919	299217	237314	451226
	Production (Ton)	3497	19244	16223	30730	16550	13836	26991
Stinging nettle	Area (Decare)	3	3	3	0	5	5	5
	Production (Ton)	0.42	0.42	0.42	0	1	1	1

Sage tea	Area (Decare)	54	30	130	536	3681	4123	3951
	Production (Ton)	7	4	19	80	411	557	428
Rose (Oil)	Area (Decare)	30832	28012	28359	28243	29753	33277	34205
	Production (Ton)	10225	10769	10831	9483	12267	13372	14773
Lavender	Area (Decare)	509	709	3189	3218	5700	6606	8684
	Production (Ton)	123	105	297	400	747	845	1040

Table 2. Production amounts (ton) of some medicinal and aromatic plants in the world in 2017 [27].

Items	**Area**	**Production amount (ton)**
Anise, badian, fennel, coriander	India Mexico Iran	646 000 132 565 64 788
Cinnamon (canella)	Indonesia China Viet Nam	87 130 79 486 37 126
Garlic	China India Bangladesh	22 160 465 1 693 000 425 401
Ginger	India China Nigeria	1 070 000 557 303 349 895
Jojoba seed	Mexico	143
Maté	Brazil Argentina Paraguay	619 003 290 950 105 005
Mustard seed	Nepal Canada Russian Federation	159 710 121 600 98 319
Pepper (piper ssp.)	Viet Nam Indonesia Brazil	252 576 87 029 79 371
Quinoa	Peru Bolivia Ecuador	78 657 66 792 1 286
Vanilla	Madagaskar Indonesia China	3 227 2 402 662

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3. PROPAGATION OF PLANTS CONTAINING SECONDARY METABOLITES WITH PLANT TISSUE CULTURE METHODS

It is important to have knowledge the chemistry and biology of the active substances of medicinal and aromatic plant species that have economic value today and to produce these plants. With the increase in the consumption of natural products all over the world, there are difficulties in obtaining sufficient amount of herbal raw materials. Plant metabolites can be isolated from plants grown in nature but some problems are also encountered in obtaining secondary metabolites from plants grown in natural environment. Some of these problems can be listed as climatic factors, the risk of extinction of the plant generation, the inability to obtain sufficient secondary metabolites and inability to provide a drug of high quality and efficiency [28-30,31]. Important medicinal plants can be propagated by plant tissue culture methods. In addition, plant tissue cultures are a promising strategy especially in rare or endangered species or in plants producing secondary metabolites that are difficult to propagate.

In recent years, interest in plant tissue cultures has increased in various countries of the world. Advances in this technique contribute to the solution of problems related to subjects such as physiology, biochemistry, cytology, genetics and molecular biology in plants [17,18,28,30,32-33].

Plant tissue culture methods have great industrial importance in plant propagation, conservation of plant resources and production of secondary metabolites in recent years. This methods offers new and sustainable opportunities in solving numerous problems in the field of medicinal plant breeding and conservation biology [5,23,28,34,35]. Secondary metabolites are compounds that are not a direct metabolism product in plants but appear as by-products, are produced by plants and do not have a direct relationship with the plant's primary metabolism, but are produced to the organism advantage and essentially have very important roles in plants. Secondary metabolites defend plants against microorganisms, insects, herbivores and even other plants. In addition, secondary metabolites have an ecological role in nature. They function as substances that attract animals that assist in pollination or seed distribution [2,32,34,36,37]. Plants, used in pharmacy, agriculture, food additives and as bio pesticide, are main materials constitute an important source of secondary metabolites.

In previous years, secondary metabolites were obtained by isolating from plants. After the 1970s, cell cultures followed by tissue culture methods began to be used to produce secondary metabolites under in vitro conditions. Plant secondary metabolites are used directly or indirectly in many industries, especially in the pharmaceutical and food industry [5,32,36,38,39]. With the rapid increase in the world population, excessive pressure on the existing arable lands has caused disappearance of the natural habitats and it has become increasingly difficult to obtain plant-derived compounds. Plant secondary metabolites can be produced by in vitro callus induction, cell suspension cultures and organ cultures. As an alternative for secondary metabolite production, plant cells and organ cultures have been developed. The advantages of production of secondary metabolites with plant tissue culture is desired production at desired time, uniform quality product in a short time, independent production throughout the year regardless of climatic conditions, conservation of wild species at risk of extinction and production of novel compounds. New plant compounds that are not normally found in natural populations of certain species can also be isolated by plant tissue culture methods. Sustainability of important medicinal plant species for future can be achieved by reducing the dependence on the natural population by producing these compounds by in vitro culture methods. [30,40-43].

Commercial production of some secondary metabolite products from plant cell and tissue cultures has successfully been carried out recently. Therefore, the selection of a suitable species and organs is necessary for induction of in vitro callus, cells or organs. Cell and organ cultures are started by selecting a mother plant with a high amount of secondary metabolites. In fact, plant tissue cultures are an alternative and effective technique in the production of bioactive compounds, and the amount of secondary metabolites produced in this technique is higher than the mother plant [5,30,38,41,42,44]. In a study by Kumar et al. [45], it was determined that the amount of secondary metabolites in the plantlets grown *in vitro* of *Swertia chirayita* plant is higher than *in vivo* plantlets. *Fritillaria unibracteata* was rapidly propagated directly from small cuttings of the bulb by organ culture technique. Compared to natural wild growth conditions, the growth rate was about 30-50 times higher and alkaloid and useful microelements content were higher than these found in wild bulbs [46]. Thus, the controlled production of secondary metabolites with plant cell and tissue cultures, are numerous benefits in is highly promising [28].

4. STRESS FACTORS IN PLANT TISSUE CULTURES

Secondary metabolite production from medicinal plants has accelerated in the last decade. In land conditions, both internal factors such as the genotype, plant organ and age of the plant, and external factors such as photoperiod, temperature, soil type, light intensity and wavelength, and amount of water available and climatic conditions affect the concentration and content of compounds obtained from medicinal plants. The reaction of plants to stress factors varies depending on the intensity and duration of stress [30,36,47,48]. The above-mentioned factors may also affect the content of medicinal plants produced *in vitro*. In a study by Kapoor et al. [49], it was revealed that the quality of light in callus cultures of the medicinal plant *Rhodiola imbricata* is an important factor in the growth rate of callus biomass and in the production of industrially important secondary metabolites. Biomass and alkaloid production of *Catharanthus roseus* increased by the inclusion of 200 mg/L tryptophan or phenylalanine as a nitrogen source in the B5 nutrient medium with a pH of 5.82 [50].

The response of plants to abiotic stress factors consists of four stages in general: 1- Initial alarm phase, 2- Acclimation phase, 3- Maintenance phase and 4- Exhaustion phase (Figure 1) [51]. Plant response to stress is a dynamic process that is dependent on stress intensity and stress duration. Plant's response to stress can be distinguished several stage: *An initial alarm phase*, when stress in plant causes a shock effect, stress tolerance level of plant decreases and stress-responsive signalling pathways are stimulated; biosynthesis of various stress-protective proteins takes place and plant stress tolerance level increases during *acclimation phase*; homeostasis is maintained and plant stress tolerance level remains constant during the *maintenance phase*; plant stress tolerance level declines during *exhaustion phase* and if stress application takes too long, the plant maintains stress-induced homeostasis will fail (Figure 1).

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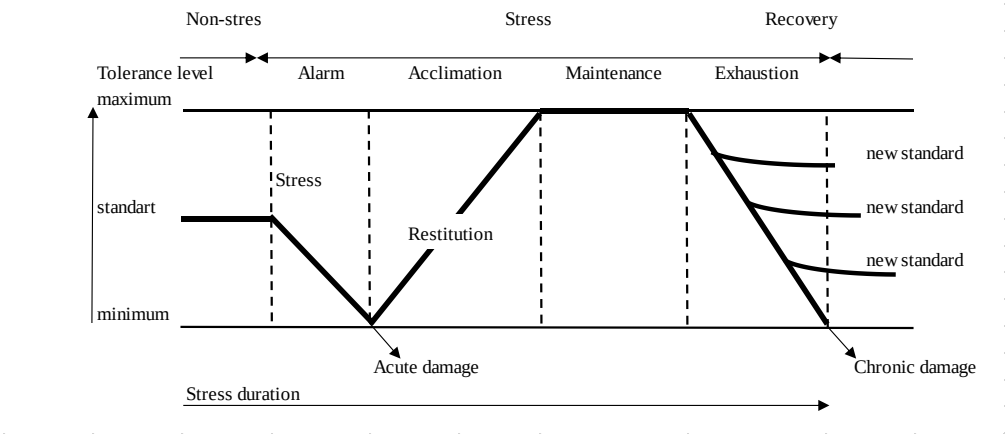

Figure 1. A generalized scheme of plant responses to abiotic stress factors [49].

Secondary metabolites production, role in plant defence and whose synthesis is triggered by stress factors such as climatic factors, drought, light and microbial activities, can be increased in plant tissue and cell cultures by factors defined as stimulants (elicitor). Elicitors can be used for triggering the secondary metabolic pathway in plant cell. Elicitors can be divided into biotic and abiotic. For this purpose, biotic and abiotic stimulants indicated in Figure 2 can be used. Abiotic stimulants consist of substances of non-biological origin and can be grouped as physical, chemical and hormonal factors. Biotic stimulants are substances of biological origin like are polysaccharides, proteins, glycoproteins, bacteria, fungi and yeasts [52-57].

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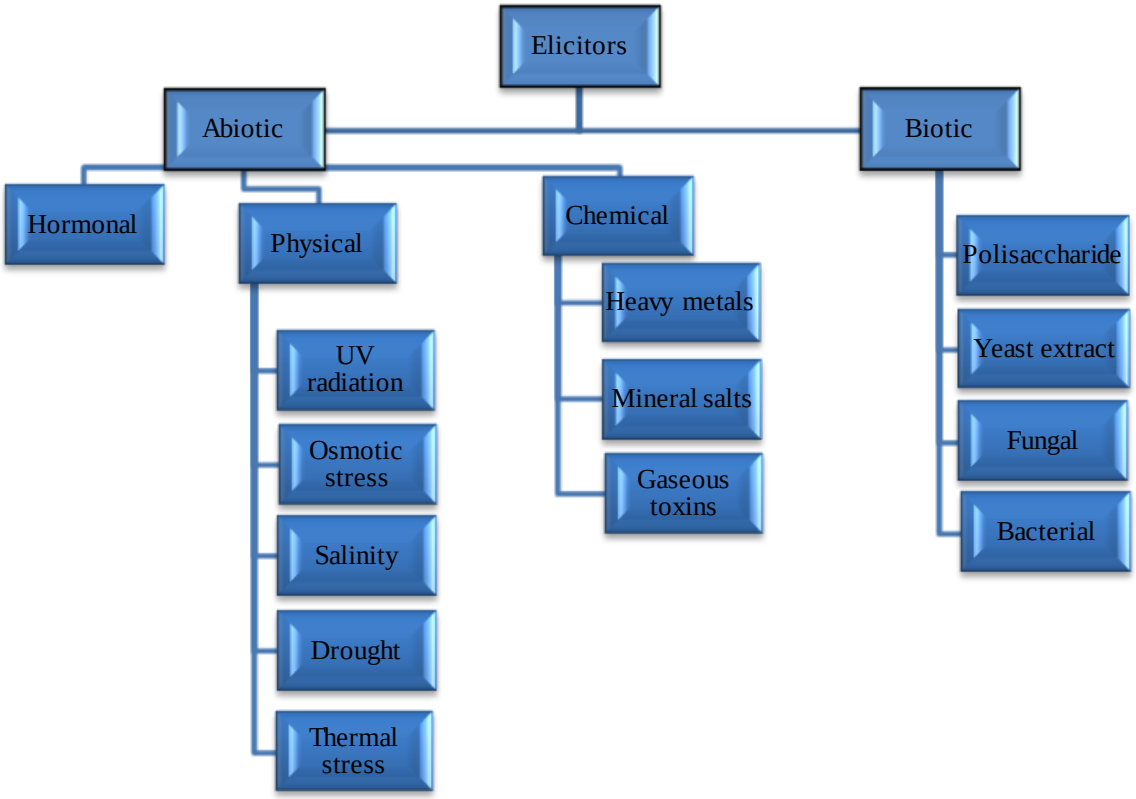

Figure 2. Classification of biotic and abiotic elicitors in plants [50].

Yamaner et al. [58] indicated that mannan and pectin added to the MS nutrient medium stimulate of hypericin biosynthesis in *Hypericum adenotrichum* seedlings. Accordingly, the production of pseudohypericin was increased by 2.8 times and the production of hypericine was increased by 1.7 times with the treatment of varying levels of mannan. Pectin treatment stimulated pseudohypericin production up to 4.8-fold and hypericine production up to 2.7-fold. It was revealed by the study that these elicitors can be used in the secondary metabolites production in *Hypericum adenotrichum* plant. Also, Yu et al. [59] indicated that jasmonic acid is an effective elicitor for secondary metabolite production. Thus in their study 1.0-5.0 mg/L jasmonic acid, strongly promoted total ginsenoside content and ginsenoside productivity. *In vitro* cultures of *Merwilla plumbea*, the nutrient medium containing 100 mg/L yeast extract (YE) stimulated the accumulation of total phenolic substances in the roots, followed by 100 mg/L yeast malt broth (YMB) in shoots [60]. Açıkgöz et al. [61] reported that different concentrations of methyl jasmonate and salicylic acid treatments increased the the camphor and phenolic compounds accumulation. The highest amount of camphor accumulation was seen in cells treated with 100 µM MeJA (0.3449 µg/g) and 50 µM SA (0.3816 µg/g). Sutini et al. [62]

demonstrated that with the addition of cobalt metal ion elicitors to the nutrient medium, the secondary metabolite of cinnamic acid was obtained in the *Camellia sinensis* by 11.9%.

5. CONCLUSION

There are many plant species with medicinal value in the world. Collection and export of some plant species for medicinal purposes destroy the flora and threaten some of the plant species [63]. Demand for herbal products has increased rapidly in past three decades due to the low side effects of herbal medicines [64]. Medicinal and aromatic plants are herbal sources preferred by the majority of the world population for a healthy life and are also used as a preventive and therapeutic treatment [65]. Plant tissue cultures methods play an important role in obtaining secondary metabolites from medicinal and aromatic plants and meeting the growing demands of the pharmaceutical industry in an environmentally friendly way. Plant tissue culture techniques are often used for large-scale production of secondary metabolites in plant species that are often difficult to cultivate or of medicinal importance. However, tissue culture methods also play an important role in conserving biodiversity and propagating rare endangered plant species [66].
In vitro propagation has an important potential for the production of high quality medicinal products. Despite the fact that endangered medicinal plants are protected by *in vitro* propagation, many plant species of medicinal importance are unconsciously exploited by the pharmaceutical industry. In order to reduce the pressure caused by excessive collection of medicinal plants from nature and to produce metabolites effectively, these plant species should be cultured quickly and studies on *in vitro* production should be increased.

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