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The Studies on Seed Germination and *in Vitro* Cultures of *Salvia* L. Species from Turkish Flora

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Abstract

Turkey has a rich flora in terms of plant biodiversity, and economically important *Salvia* species because of medicinal properties are in danger of extinction. To prevent the extinction of *Salvia* species is important to solving the problems of seed germination and micropropagation. In this review, *in vitro* studies conducted on *Salvia* species from Turkish flora were listed by specifying danger categories. Seed germination studies carried out 18 *Salvia* species [*S. candidissima* Vahl., *S. cyanescens* Boiss. & Balansa (endemic/LR-lc), *S. dichroantha* Stapf (endemic/LR-lc), *S. fruticosa* Mill. (VU), S. *macrosiphon* Boiss., *S. microstegia* Boiss. et Bal., *S. nemorosa* L., *S. officinalis* L., *S. pomifera* ssp. *pomifera* L. (VU), *S. sclarea* L., *S. siirtica* Kahraman, Celep & Doğan sp. nov. (endemic/CR), *S. smyrnaea* Boiss. (endemic/EN), *S. tomentosa* Mill., *S. verbenaca* L., *S. verticillata* L., *S. virgata* Jacq., *S. viridis* L.]. *In vitro* culture studies were conducted on 16 *Salvia* species [*S. aethiopis* L., *S. albimaculata* Hedge and Huber-Morath (endemic/VU)-unsuccessful result, *S. cadmica* Boiss. (endemic/LR-lc), *S. candidissima* Vahl ssp. *occidentalis* Hedge, *S. chrysophylla* Stapf (endemic/LR-cd), *S. cryptantha* Montbret et Aucher ex Bentham (endemic/LR-lc), *S. euphratica* Montbret et Aucher ex. Bentham var. *euphratica* (endemic/LR-cd)-unsuccessful result, *S. fruticosa* (VU), *S. nemorosa, S. nydeggeri* Hub.-Mor.(endemic/EN)-unsuccessful result, *S. officinalis, S. sclarea, S. tomentosa, S. verticillata* ssp. *verticillata, S. virgata, S. viridis*]. In this review has been reported up to now *in vitro* cultures have been carried out 26 of 99 *Salvia* species, has clearly shows that new protocols should be established to protect endemic and threatened *Salvia* species.

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1. INTRODUCTION

Plant species in Turkey, which has a rich flora in terms of plant diversity, have been grouped in 145 families. Labiatae (syn. Lamiaceae) family, of which *Salvia* genus is a member, is one of the richest families in point of the number of species, especially the number of endemic species (İpek and Gürbüz, 2010). *Salvia* genus, which has about 1000 species in the world (Kahraman *et al*., 2011), has 99 species, 8 subspecies and 6 varieties (106 taxon) in Turkey (Tursun, 2019; Kayıkçı and Oğur, 2020). 56 of these species in Turkey is endemic (endemism percentage 56.5%) (İpek and Gürbüz, 2010) and the total number of endemic species are 57 with the *S. siirtica* described in 2011 (Kahraman *et al*., 2011).

Like other members of Lamiaceae family, *Salvia* species are also medicinal and aromatic plants, and rich in essential oils which have been used in food, perfumes, cosmetics and pharmaceuticals (Öğütçü *et al*., 2008; Tosun *et al*., 2009; Durgha *et al*., 2015). Many and varied useful secondary metabolites such as terpenoid, phenolic compounds, and essential oils have

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been isolated from the aerial parts of sage plants (Erişen *et al*., 2020). Essential oil compounds obtained from sage plants is a complex mixture of monoterpenes, sesquiterpenes and alcohols with their oxygen derivatives, aldehydes, esters, ethers, ketones, phenols and oxides (Elmas and Elmas, 2021). *Salvia* species contain many bioactive compounds that can be divided into mainly four groups: monoterpenes, diterpenes, triterpenes and phenolic components. The most common monoterpenes include: α- thujone and β-thujone, 1,8-cineole, and camphor; diterpenes include: carnosol, carnosic acid, rosmadial and manool; triterpenes include: oleanolic and ursolic acids. The phenolic components can be divided into two groups: phenolic acids (caffeic, vanillic, ferulic and rosmarinic acids) and flavonoids (luteolin, apigenin, and quercetin). Sesquiterpene α-humulene and viridiflorol are additionally present in the extracts of *Salvia* species (Jakovljević *et al.,* 2019). Especially, *S. officinalis* is one of the richest sources of antioxidants such as rosmarinic acid, caffeic acid, carnosic acid and oligomers of caffeic acid with multiple catechol groups (Dastanpoor *et al*., 2013). Various compounds from the different parts of *Salvia* plants are identified as over 160 polyphenols, comprising an array of phenolic acids and flavonoids (Lopresti, 2017). The gas chromatography-mass spectrometry (GC/MS) analysis of essential oil extracted from *S. officinalis* was reported to the presence of 49 components with camphor (25.14%), α- thujone (18.83%), 1,8-cineole (14.14%), viridiflorol (7.98%), β-thujone (4.46%) and β-caryophyllene (3.30%) as the major components (Sharma *et al*., 2019). *S. albimaculata, S. potentillifolia* Boiss. & Heldr. ex Bentham and *S. nydeggeri* from Soutwest Anatolia in Turkey were determine their antioxidant properties and phenolic compounds using the ultra performance liquid chromatography. Caffeic acid (3582.8 ± 2.5 µg/g, 2956.5 ± 4.6 µg/g and 2457.7 ± 3.1 µg/g) and 3,4-dihydroxy benzoic acid (1846.2 ± 3.1 µg/g, 2019.1 ± 2.2 µg/g and 1901.3 ± 1.5 µg/g) were found to be in the highest concentrations in *S. potentillifolia, S. albimaculata* and *S. nydeggeri*, respectively. Total amounts of phenolics and flavonoids were obtained highest in ethyl acetate extracts of samples. Antioxidant activity of *S. potentillifolia* was found to be higher than the other studied *Salvia* species (Kivrak *et al*., 2019).

So far published literature reported that the essential oils of *Salvia* species have showed the various pharmacological properties such as antioxidant, antibacterial, anti-neurodegenerative, anti-enzymatic (anticholinesterase, anti-urease, anti-tyrosinase, anti-elastase), antidiabetic and anti-tumour activities (Aşkun *et al*., 2010; Uysal *et al*., 2021). *Salvia* species growing in Turkey has been commonly consumed among the population as a diuretic, cold remedy, stomach relaxant, bloating remedy and tonic drink (Yilar *et al*., 2018). *Salvia* species in flora of Turkey have been used against various diseaes such as high fever, rheumatic pain, stomachache, peptic ulcer, urinary inflammations, catarrh, cold, wounds, flatulence, constipation, wards, sunstroke, and hemorrhage in Turkish folk medicine (Ustun *et al*., 2016; Dumanoğlu and Mokhtarzadeh, 2020).

Due to global warming, extreme drought, salinization, deforestation, erosion, human pressure, overgrazing, air-soil-water pollution, the unconscious over-collecting of economically valuable sage plants from nature is in danger of extinction this plant. Especially all of the endemic *Salvia* species are in the threatened category. 12 species of the genus *Salvia* (S. *anatolica* Hamzaoğlu & A. Duran, *S. ballsiana* (Rech.f.) Hedge, *S. freyniana* Bornm., *S. hedgeana* Dönmez, *S. marashica* A. İlçim, F. Celep & Doğan, *S. odontochlamys* Hedge, *S. pseudeuphratica* Rech., *S. quezelii* Hedge & Afzal-Rafii, *S. sericeo-tomentosa* Rech.f., *S. siirtica*, *S. tigrina* Hedge & Hub.-Mor., *S. vermifolia* Hedge & Hub.-Mor.) are critically endangered (CR) and 14 species (*S. adenophylla* Hedge & Hub.-Mor, *S. albimaculata*, *S. aytachii* Vural & N. Adıgüzel, *S. cedronella* Boiss., *S. cerino-pruinosa* Rech. var. *elazigensis* A.Karaman, F.Celep & Dogan, *S. cilicica* Boiss. & Kotschy, *S. ekimiana* F. Celep & Doğan sp. nova, *S. eriophora* Boiss. & Kotschy ex Boiss., *S. halophila* Hedge, *S. kronenburgii* Rech.f., *S. modesta* Boiss., *S. nydeggeri*, *S. smyrnaea*, *S. tobeyi* Hedge) are in endangered (EN) category.

Besides, since especially *S. fruticosa* (syn. *S. triloba*) and *S. cryptantha*, *S. multicaulis* Vahl, *S. sclarea* and *S. tomentosa* are also widely traded, they carry the risk of being endangered category (İpek and Gürbüz, 2010).

Conservation strategies include in-situ conservation, ex-situ conservation and cultivation studies. Within the scope of ex-situ conservation strategies, using plant biotechnology and *in vitro* techniques presents some important advantages, these techniques are in conservation of species that are especially under threatened of extinction and/or endemic (Bürün, 2021). "National Biologic Diversity Strategy and Action Plan" published in 2007 by Ministry of Environment and Forestry in Turkey also suggests to grow endangered species and produce them under control (Anonymous, 2007). To do this, it is necessary to collect seeds of that species first, then to establish seed germination protocol and determine the conditions for reproduction by seed as well as to multiply with other propagation techniques and do other culture studies. Therefore within ex-situ conservation strategies, propagation studies of natural species has been important in terms of protection of genetic diversity. *In vitro* techniques are being used in secondary metabolite production and breeding besides conservation and propagation of *Salvia* species which is a valuable genus from the medicinal and aromatic characteristics (Surgun-Acar and Bürün, 2017). *In vitro* culture of medicinal *Salvia* species for the purpose of extraction of bioactive constituents may face certain limitations such as climate, season, water availability, various environmental stresses, diseases, pests and shortage of naturally growing plants (Ghanbar *et al*., 2016). These difficulties and challenges such as the differences in terrain and climate conditions, low metabolite yield and quality and more labor needed a new biotechnological approaches in order to enable more economic, higher metabolite yield and quality compared to the conventional methods (Demirci *et al*., 2015). Therefore, *in vitro* cell and organ culture techniques have emerged as an alternative way to plant growing under field conditions for secondary metabolite production (Çalışkan *et al*., 2019).

An important priority in efforts to conserve rare species is their seed germination. Because germination and seedling emergence are the most critical phases in the plant life cycle (Javaid *et al*., 2018). However, the main global problem of *Salvia* species has been the germination of their seeds and so far an integral solution has not been reported for this (Abdollahi *et al*., 2012a; Abdollahi *et al*., 2012b). The aim of this review article is to review the seed germination and *in vitro* culture studies of the economically important *Salvia* genus in Turkey and to evaluate them in terms of ex-situ conservation strategies.

1.2. Seed Germination Studies on *Salvia* Species in Flora of Turkey

The physiological events related to seed germination are the first step of plant life (Tursun, 2019). Seed germination studies of rare, endangered and endemic species have a great importance in determining in situ and ex situ conservation strategies. Seed germination is the only way to preserve genetic diversity, and many plant species are at risk of being extinct. Therefore, investigations on the germination abilities of endangered plant seeds are important to conservation for the next generation (Subaşı and Güvensen, 2010).

Seed germination studies of *Salvia* species has been carried out only in 18 species (4 of which is endemic) and the first has been reported in *S. fruticosa* by Thanos and Doussi (1995). *Salvia* genus has 99 species in Turkish flora. There are 12 species in critically endangered (CR), 14 species in endangered (EN), 15 species in vulnerable (VU), 17 species in the least concern (LR-lc), 2 species in near threatened (LR-nt) categories, and 1 species (*S. reeseana* Hedge & Hub.-Mor.) is data deficient (DD). Germination percentages has been found between 21.25% (*S. siirtica*) and 97% (*S. verbenaca*) in 18 species studied, including 1 species (*S. siirtica*) in endemic/CR, 2 species (*S. fruticosa*, *S. pomifera* L. ssp. *pomifera*) in VU, 1 species (*S. smyrnaea*) in endemic/EN, 2 species (*S. cyanescens, S. dichroantha*) endemic/LR-lc and another 12 species not endangered categories. As it is seen in Table 1, few studies have been

conducted out on seed germination, and seed germination has been investigated only in one (*S. siirtica*) of 12 species of which is in the CR category. Seed germination has been studied only on *S. smyrnaea* from 13 species in the EN category, and 66.6% germination has been achieved with some applications.

Table 1. Seed germination studies in *Salvia* species in flora of Turkey (The species are listed in an alphabetic order).

Species	Endemic/ Danger status	Germination conditions and/or Pre-treatments	Germination percentage	Reference
S. candidissima	–/–*	Gibberellic acid (0, 250, 500, 750 mg l^{-1}) and temperature (5, 10, 15, 20, 25, 30 and 35°C) treatments	4.4%	(Dadaşoğlu and Özer, 2014)
S. cyanescens	+/LR(lc)*	Pretreated for 5 minutes at -5°C / top of paper at 25°C	78%	(Yücel and Yilmaz, 2009)
S. dichroantha	+/LR(lc)*	In darkness, at 20°C	80%	(Thanos and Doussi, 1995)
		Storage at 4°C for 10 days, then soaking in 500 ppm Gibberellin solution	50.2%	(Özcan *et al*., 2014)
		Polymer coating+GA_3 Application GA_3 at 25°C	85% 82%	(Sönmez *et al*., 2019)
S. macrosiphon	–/–*	Gibberellic acid [0 (control), 100, 150 and 200 mg l^{-1}]	62%	(Abdollahi *et al*., 2012a)
		Gibberellic acid(150 mg l^{-1}) under drought stress (PEG)	significant correlation between germination and drought	(Abdollahi *et al*., 2012b)
S. microstegia	–/–*	Gibberellic acid (0, 250, 500, 750 mg l^{-1}) and temperature (5, 10, 15, 20, 25, 30 and 35°C) treatments	50%	(Dadaşoğlu and Özer, 2014)
S. nemorosa	–/–*	Gibberellic acid (0, 250, 500, 750 mg l^{-1}) and temperature (5, 10, 15, 20, 25, 30 and 35°C) treatments	50%	(Dadaşoğlu and Özer, 2014)
S. officinalis	–/–*	Seed priming (10, 20, 30°C for 0, 12, 24, 48 h.)	77.5%	(Dastanpoor *et al*., 2013)
		Mercury (II) chloride (0.1%) for 5, 10 minutes or sodium hypochlorite (30%) for 10, 15 minutes	50%	(Yaschurevskaya and Cherednichenko, 2017)
S. pomifera ssp. *pomifera*	–/VU*	In darkness, at 15°C	70%	(Thanos and Doussi, 1995)
		At 25°C	42%	(Joshi and Pant, 2010)
S. pomifera		In MS medium containing 80 ppm chitosan and pH:7-8	85.8%-86.7%	(Ghanbar *et al*., 2012)

		Storage at 4°C for 10 days, then soaking in 500 ppm Gibberellin solution	47.6%	(Özcan *et al.,* 2014)
S. sclarea	–/–*	In paper at 2 days open in room temparature and then incubated at 20°C under continuous dark	89.3%	(Kumar and Sharma, 2012)
		Mercury (II) chloride (0.1%) for 5, 10 minutes or sodium hypochlorite (30%) for 10, 15 minutes	90%	(Yaschurevskaya and Cherednichenko, 2017)
S. siirtica sp. nov.	+/CR**	Stratification (28 day in fine sand maintaining in refrigerator of 4°C)	21.2%	(Arslan *et al*., 2017)
S. smyrnaea	+/EN*	Stratification (at 5°C, 45 days), in dark (25/15°C), 250 ppm GA_3 application	66.6%	(Subaşı and Güvensen, 2010)
S. tomentosa	–/–*	Storage at 4°C for 10 days, then soaking in 500 ppm Gibberellin solution	39.6%	(Özcan *et al*., 2014)
S. verbenaca	–/–*	Scabbing treatment	53%	(Khakpor *et al*., 2011)
		12 hours light/12 hours dark; the complete darkness regime of 24 hours dark; darkness and day/night temperatures: 30/20˚C; 20/15˚C; 25/12˚C	97%	(Javaid *et al*., 2018)
S. verticillata	–/–*	Gibberellic acid (0, 250, 500, 750 mg l^{-1}) and temperature (5, 10, 15, 20, 25, 30 and 35°C) treatments	32.3%	(Dadaşoğlu and Özer, 2014)
		Giberellic acid (2000 ppm) in dark at 26/16°C	74%	(Tursun, 2019)
		At -80°C for 4 days, in dark at 20±1°C	57-67%	(Tursun, 2020)
S. virgata	–/–*	Gibberellic acid (0, 250, 500, 750 mg l^{-1}) and temperature (5, 10, 15, 20, 25, 30 and 35°C) treatments	44%	(Dadaşoğlu and Özer, 2014)
S. viridis	–/–*	Mercury (II) chloride (0.1%) for 5, 10 minutes or sodium hypochlorite (30%) for 10, 15 minutes	90%	(Yaschurevskaya and Cherednichenko, 2017)

*(İpek and Gürbüz, 2010), **(Kahraman *et al*., 2011). IUCN: The International Union for Conservation of Nature. IUCN Categories: CR-Critically Endangered, EN-Endangered, VU-Vulnarable, LR-Lower Risk LR(cd)-Conservation Dependent, LR(lc)-Least Concern. MS: Murashige-Skoog (1962)

1.3. *In vitro* Cultures on *Salvia* Species in Flora of Turkey

Plant biotechnology allows efficient, fast, large scale and easily production of plant materials and their isolated compounds. *In vitro* cultures offer many advantages such as independence from climatic and geographical conditions, the possibility of efficient propagation of species in a short time from a small quantity of explants as a primary material (Grzegorczyk-Karolak *et al*., 2021). *Salvia* species is propagated different parts such as shoot tip, nodal segment, leaf, hypocotyl of the sterile plants via seeds germination. In nature, after

Salvia seeds remain dormant for a long time, they germinate very slowly. Therefore, *in vitro* cultures for large scale multiplication would be a viable option, and several medicinal plants are considered an effective tool to multiply difficult to propagate, useful, rare and endangered species for different commercial purposes (Jafari *et al*., 2017a).

Table 2 shows that *in vitro* culture applications on *Salvia* species from Turkish flora were investigated after the 1999s. *In vitro* culture studies have been encountered only in 16 species of the ones that exist in Turkey and successful results have been obtained in 13 species. Mostly studied species are *S. sclarea* and *S. fruticosa. S. sclarea* is not an endemic or threatened with extinction species but it is heavily traded. *S. fruticosa,* on the other hand, is a commercially traded species in the VU category. While there is only eight study for secondary metabolite production of *S. cryptantha* (endemic/LR-Ic) and *S. tomentosa*, which are traded, there is not an *in vitro* culture study on *S. multicaulis* (Table 2).

Table 2. Seed germination studies in *Salvia* species in flora of Turkey (The species are listed in an alphabetic order).

Species	Endemic / Threatened position*	Explant	Medium	Goal/Result	Reference
S. aethiopis	–/–	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
		Hypocotyls	MS	Production of secondary metabolites	(Vulganová *et al*., 2019)
S. albimaculata	+/EN	Nodal explant and shoot tip	MS	Micropropagation/ unsuccessful	(Uyanık, 2017)
S. cadmica	+/LR(lc)	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
S. candidissima ssp. *occidentalis*	–/–	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
S. chrysophylla	+/VU	Nodal explant and shoot tip	MS	Micropropagation/ regeneration, rooting and acclimatizion were achieved	(Uyanık, 2017)
S. cryptantha	+/LR(lc)	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
S. euphratica var. e*uphratica*	+/LR(lc)	Nodal explant and shoot tip	MS	Micropropagation/ unseccessful	(Uyanık, 2017)
S. fruticosa	–/VU	Leaf (from young plants)	MS	Somatic embryogenesis and rosmarinic acid accumulation	(Kintzios *et al*., 1999)
		Shoot tips (from *in vivo* and *in vitro* seedlings)	MS and B_5	Callus, cell suspension and root cultures, rosmarinic acid accumulation	(Karam *et al*., 2003)

		Shoot tips (from *in vitro* seedlings)	MS, NN, B_5	Micropropagation and accumulation of essential oils/A protocol was developed for micropropagation and essential oil in microshoots was high	(Arikat *et al*., 2004)
		Cell cultures obtained leaf	LS	The production variability of different metabolites (phenols and sterols) from cell suspension culture	(Kümmritz *et al*., 2014)
S. nemorosa	–/–	Shoot tip, leaf lamina and petiol	MS	Micropropagation/ Protocol described	(Skała and Wysokińska, 2004)
S. nydeggeri	+/EN	Nodal explant and shoot tip	MS	Micropropagation/ unseccessful	(Uyanık, 2017)
S. officinalis	–/–	Leaf explants	MS	Regeneration, micropropagation, callus cultures and somatic embryogenesis	(Ioja-Boldura *et al*., 2010)
		Shoot tip	MS	Synthetic seed production	(Grzegorczyk and Wysokińska, 2011)
		Cell cultures obtained leaf	LS	The production variability of different metabolites (phenols and sterols) from cell suspension culture	(Kümmritz *et al*., 2014)
		Nodal segments	MS	Efficient method for micropropagation to evaluate flavonoid content and antioxidant capacity	(Petrova *et al*., 2015)
		Leaf and internode	MS	Influence of putrescine and thidiazuron on *in vitro* organogenesis	(Jafari *et al*., 2017a)
		Leaf and internode	MS	Indirect organogenesis and plant regeneration	(Jafari *et al*., 2017b)
S. sclarea	–/–	Immature zygotic embryo and cotyledons	MS	Organogenesis/ Establish a plant regeneration system	(Liu *et al*., 2000)
		Leaf (35-day old seedlings)	MS	Investigate the process of transformation/*In vitro* induction of crown galls	(Khawar *et al*., 2003)
		Axillary and apical buds	MS	Micropropagation	(Mascarello *et al*., 2006)

		(from adult plants)			
		Nodal segments	MS	Synthetic seed production	(Durgha *et al*., 2015)
		Shoot tips and axillary node (from16-day-old seedlings)	MS	Micropropagation and conservation/ this work describes successful regeneration	(Ghanbar *et al*., 2016)
		Hypocotyls	MS	Production of secondary metabolites	(Vulganová *et al*., 2019)
		Nodal segments	MS	Micropropagation/ Protocol described	(Erişen *et al*., 2020)
		Leaves and/or callus	MS	to investigate chemical properties and biological activities	(Saruhan-Fidan *et al*., 2021)
S. tomentosa	–/–	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
S. verticillata ssp. *verticillata*	–/–	Seed	MS	Callus induction for utilization in secondary metabolite production	(Rezaeieh *et al*., 2012)
S. virgata	–/–	Cell cultures obtained leaf	LS	The production variability of different metabolites (phenols and sterols) from cell suspension culture	(Kümmritz *et al*., 2014)
		Nodal explants	MS	Phenolic acids production in shoot cultures	(Dowom *et al*., 2017)
S. viridis	–/–	Hairy root cultures	MS	Production of polyphenolic compounds	(Grzegorczyk-Karolak *et al*., 2018)
		Shoot and/or callus cultures	–	to investigate their potential for the accumulation of phenolic compounds	(Grzegorczyk-Karolak *et al*., 2019)
		In vitro shoot culture	MS	to investigate the effects of purine-type cytokinins on the polyphenolic compounds accumulation	(Grzegorczyk-Karolak *et al*., 2020)
		Shoot	MS	Synthetic seed production	(Grzegorczyk-Karolak *et al*., 2021)

*(İpek and Gürbüz, 2010). MS: (Murashige and Skoog, 1962), NN: (Nitsch and Nitsch, 1969), B_5: Gamborg's medium, LS: Linsmaier and Skoog.

The majority of *in vitro* studies on *Salvia* species are micropropagation, organogenesis, plant regeneration, callus induction, secondary metabolite production and synthetic seed production. The micropropagation of *S. albimaculata* and *S. nydeggeri* which is endemic in EN category has been studied; however these efforts have not been successful. Furthermore, successful results can not also be obtained from the study on micropropagation of *S. euphratica* var. *euphratica* which is endemic in LR-lc category (Table 2).

4. CONCLUSION

Turkey exhibits great wealth in terms of plant species number, biodiversity and endemism as a result of different geographical factors such as climate, altitude, soil structure. The genus *Salvia*, which has a high endemism rate, has medicinal and aromatic properties such as antibacterial, antifungal, antiviral, antiseptic, analgesic, antidiabetic. However, many *Salvia* species are under threat of extinction because of unconscious, uncontrolled and excessive collection. It is essential to implement appropriate ex-situ strategies to conserve and grow especially endemic or threatened species. In ex-situ conservation strategies will be managed according to the information about the species, thus success in ex-situ conservation will be achieved. For this reason, seed germination conditions of the species should be determined first and propagation by seed should be investigated. In addition, for species that cannot be produced by seeds or even if they can be propagated by seeds, their production by *in vitro* techniques should be investigated and appropriate micropropagation protocols should be established. In order to protect the plant diversity of Turkish flora, it is essential to carry out these studies and to implement conservation strategies. It is important to use plant biotechnology approaches, especially *in vitro* cultures, in the conservation of threatened plant species. Some conservation strategies for endangered and traded *Salvia* species in Turkey must be applied immediately. Only 26 species out of 99 species have been established on seed germination and *in vitro* culture studies. Compared to the total number of *Salvia* species found in Turkey, the rate of species studied *in vitro* is very low, at 26%. Conservation strategies need to focus on unstudied species and determine appropriate protocols urgently.

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Author Contribution Statement

Emel Yilmaz Gokdogan: Writing, editing, validation. **Betul Burun:** Idea, design, writing.

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