Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Callus Production in Geranium (*Pelargonium quercetorum* Agnew) Growing Naturally in Türkiye

Metin KOÇAK*[1], Sibel TURAN SİRKE[2], Cansu KUZĞUN[3], Mehtap YILDIZ[4]

[1,2,3,4]Van Yuzuncu Yil University, Faculty of Agriculture, Department of Agricultural Biotechnology, Tuşba, 65080, Van, Türkiye

[1]https://orcid.org/0000-0002-8109-5245, [2]https://orcid.org/0000-0003-1233-0077, [3]https://orcid.org/0000-0002-7881-6507
[4]https://orcid.org/0000-0001-6534-5286

*Corresponding author e-mail: metinkocak@yyu.edu.tr

Article Info

Received: 31.05.2023
Accepted: 02.08.2023
Online published: 15.09.2023
DOI: 10.29133/yyutbd.1307775

Keywords

Agnew,
Callus,
Organogenesis,
Pelargonium quercetorum,
Somatic embryogenesis

Abstract: *Pelargonium quercetorum* Agnew grows naturally in the Hakkari province of Türkiye. Although *P. quercetorum* Agnew has potential use as a medicine and ornamental plant, it is especially used as a medicinal plant for the cure of various diseases by local people. *In vitro* tissue culture methods are favorable for the propagation, conservation, and breeding of medicinal plants. We aimed in this study to achieve regeneration of *P. quercetorum* Agnew from different explant types. Seeds of *P. quercetorum* Agnew were germinated *in vitro* conditions and explants were taken from these germinated sterile plantlets. Totally four different experiments, containing three of them embryogenic and one of them organogenic culture, were established to achieve regeneration in *P. quercetorum* Agnew. Leaf, petiole, cotyledon, cotyledon stalk, and root collar disc were used as explant. Different concentrations of 1-Naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic (2,4-D), 6-Benzylaminopurine (BA), 6-Furfurylaminopurine (Kinetin), 6-(γ,γ-Dimethylallylamino) purine (2iP), and Thidiazuron (TDZ) were used to induce embryogenic or organogenic regeneration. Explants were cultured in half-strength or full-strength Murashige and Skoog (MS) medium. In the embryogenic experiments, callus formation from different media ranged from 63.5% to 100%, and for explant types ranged from 39% to 100%. In the organogenic experiment, callus formation from different media ranged from 12.5% to 100%, and for explant types ranged from 71% to 93%. Also, embryo-like structures were obtained from embryogenic experiments. However, these structures could not grow more and transformed into plantlets.

To Cite: Koçak, M, Turan Sirke, S, Kuzğun, C, Yıldız, M, 2023. Callus Production in Geranium (*Pelargonium quercetorum* Agnew) Growing Naturally in Türkiye. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(3): 503-512. DOI: https://doi.org/10.29133/yyutbd.1307775

1. Introduction

Pelargonium quercetorum Agnew, most of which grows in and around South Africa, is included in the Geraniaceae family, which includes more than 220 species (Taherpour et al., 2008; Röschenbleck et al., 2014). One of the places where geranium grows naturally outside of South Africa is Anatolia. Another naturally grown geranium species was described by Agnew (1967) in the Mosul province of Iraq. This species, which was found around the Hakkari-Zap River in Türkiye, spreads in Southeastern Anatolia and is known as “Tolk” in the Gecitli village of Hakkari (Davis, 1967; Kaval et al., 2014).

The use of geranium plant, which has been used as a medicine among people for many years, is limited as an ornamental plant. Currently, naturally grown geranium plants do not have sufficient potential for use as ornamental plants. In order to increase the use of geranium as an ornamental plant and to gain a place in the ornamental plants' sector, it is necessary to develop the propagation methods of existing natural species. Ornamental plants can be propagated by seed or vegetatively by conventional methods, or by using tissue culture techniques.

In vitro, tissue culture techniques can be an effective alternative tool to classic techniques for propagation, conservation, and breeding of plants. One of the most frequently used techniques among tissue culture techniques is somatic embryogenesis. Somatic embryogenesis is the process of producing embryos from somatic tissues of the plant in a bipolar structure that retains the genotype of the parent plant. Somatic embryogenesis is one of the most effective techniques for mass vegetative propagation *in vitro* (Öktem and Yücel, 2012). These embryos consisting of somatic cells are called somatic embryos. Embryos can develop directly from somatic cells (direct somatic embryogenesis) or occur as non-embryogenic mitotic divisions (indirect somatic embryogenesis) before embryogenic structures appear (Rout et al., 2006). Significant differences are observed between species in terms of somatic embryo formation frequency, and embryo formation abilities of different genotypes and varieties within the same species are different. Somatic embryogenesis can be successfully applied in ornamental plants as well as in many plants. Somatic embryogenesis has been reported in cyclamen (Koçak et al., 2014), tulip (Bakhshaie et al., 2010), cloves (Pareek and Kothari, 2003), and begonia (Castillo and Smith, 1997).

Another propagation in tissue culture used widely is organogenesis. In many ornamental plants; *Lilium pumilum* (Zhang et al., 2016), *Camellia nitidissima* (Lü et al., 2013), *Echinacea purpurea* (Choffe et al., 2000), *Dendrobium kingianum* (Habiba et al., 2018), *Tulipa tarda* (Maślanka and Bach, 2014), *Polianthes tuberosa* (Daneshvar et al., 2022), *Chrysanthemum morifolium* (Kazeroonian et al., 2018) and *Begonia elatior* (Mendi et al., 2009) organogenesis has been reported.

Both somatic embryogenesis and organogenesis occur directly or with a callus phase. Callus is described as an undifferentiated cell mass. Callus cultures are used frequently for micropropagation, utilizing from variation and obtaining cell suspension cultures in *in vitro*. Callus is also a source for the production of secondary metabolites produced by plants for defense, protection, survival, and persistence (Altan and Duru, 2017). The utilization of callus culture has become widespread, serving as a crucial method in both experimental and commercial contexts for generating valuable therapeutic compounds. These compounds span a diverse range, including antibiotics designed to combat resilient infections, widely acknowledged anticancer agents, and medicinal nanoparticles (Benjamin et al., 2019).

There are papers reporting regeneration in the other some species of *Pelargonium*; *P. graveolens* (Sreedhar, 1999; Saxena et al., 2000; Benazir et al., 2013), *P. sidoides* (Kumar et al., 2015), *P. rapaceum* (Sukhumpinij et al., 2010), *P. radula* (Zuraida et al., 2014) and *P. odoratissimum* (Ebrahimzadeh et al., 2022). However, according to our best knowledge, there is no study in literature reporting *in vitro* regeneration or callus formation in *P. quercetorum* Agnew. Therefore, in this study, we aimed to produce callus in *P. quercetorum* Agnew by inducing embryogenic and organogenic regeneration with various plant growth regulators and different combinations-concentrations of them.

2. Material and Methods

In the study, seeds of *Pelargonium quercetorum* Agnew were collected from plants growing naturally around Hakkari. Seeds of *P. quercetorum* Agnew were sterilized and germinated in tissue culture conditions. Different parts of sterile plantlets (leaves, petioles, cotyledons, cotyledon stalks, and root collar discs) were used as initial explant for regeneration experiments.

2.1. Surface sterilization and germination of seeds

The seeds were kept in 70% ethanol for one minute afterward rinsing them in sterile distilled water. In the second step of sterilization, seeds were treated in 30% sodium hypocloride (Domestos® commercial product) for 30 minutes and rinsed with sterile distilled water until removal of sterilization solution. Before the transformation of sterile seeds into germination medium, they were dried on blotting paper. Sterile seeds were transferred to a culture tube containing ½ strength of MS and, cultured at 24 °C and under 16 hours light / 8 hours dark photoperiod.

2.2. Regeneration experiments

Four different experiments consisting of three of them inducing embryogenic regeneration and one of them inducing organogenic regeneration were established to provide regeneration in *P. quercetorum* Agnew.

Embryogenic culture 1 (E1): In this experiment leaf, petiole, cotyledon, and root collar disc were used as explant. To induce embryogenic regeneration, NAA, 2,4-D, BA, Kinetin, and 2iP were added to ½ MS medium (Table 1). Explants were cultured at 24 °C full dark condition. After four weeks from the initiation of culture and forming callus mass, subcultures were carried out to new media without hormones. In these nutrient media, explants were transferred to new culture media routinely every month.

Embryogenic culture 2 (E2): Calli obtained from embryogenic culture 1 were incubated in full-strength MS medium supplemented with 2 mg l^{-1} 2,4-D and 0.5 mg l^{-1} kinetin (Table 1). All explants were cultured at 24 °C under 16 hours light / 8 hours dark photoperiod. After calli formation, explants were transferred to a hormone-free medium and subcultured per month with the same incubation conditions.

Embryogenic culture 3 (E3): This culture was established by culturing sterile leaf, petiole, cotyledon, and cotyledon stalk in a full-strength MS medium containing 0.1 g l^{-1} myo-inositol. Also, the effect of 0,02 g l^{-1} glutamine was tested. Various concentrations of NAA, 2,4-D, and TDZ were used as hormones to induce embryogenic callus formation (Table 1). After calli formation, explants were transferred to a hormone-free medium and subcultured per month. Explants were cultured at 24°C under 16 hours light / 8 hours dark photoperiod.

Organogenic culture (O): Leaf, petiole, and root collar disc were cultured in full-strength MS medium supplemented with various concentrations of NAA, 2,4-D, BA, and kinetin (Table 1). All explants were cultured at 24 °C under 16 hours light / 8 hours dark photoperiod.

Table 1. Concentrations and types of ingredients used in regeneration experiments

	Medium	NAA mg l^{-1}	2,4-D mg l^{-1}	BA mg l^{-1}	Kin. mg l^{-1}	2iP mg l^{-1}	TDZ mg l^{-1}	Glutamine
Embryogenesis	E1-1		2					
	E1-2		2	1				
	E1-3		2		1			
	E1-4		2			1		
	E1-5	2						
	E1-6	2		1				
	E1-7	2			1			
	E1-8	2				1		
	E2		2		0.5			
	E3-1	1					0.5	+
	E3-2	2					0.5	+
	E3-3		1				0.5	+
	E3-4		2				0.5	+
	E3-5	1					0.5	-
	E3-6	2					0.5	-
	E3-7		1				0.5	-
	E3-8		2				0.5	-
Organogenesis	O1	1		2				
	O2		1	2				
	O3				2			
	O4	1			2			
	O5		1		2			
	O6					2		
	O7	1				2		
	O8		1			2		

2.3. Experimental design and statistical analysis

The experiments were established as completed randomized plots (5 replicate/petri x 4 explants). All percentage data were transformed to arcsine value. Means were compared with an analysis of variance and significant differences were determined by performing an LSD test. All data analyses were carried out with the JMP® program (SAS Institute, Cary, NC) ver. 8.00.

3. Results

In the germination culture of the seed, no infection was observed. The germination rate was 83%, which suggested sterilization method did not decrease the germination capacity of seeds significantly. Seeds started to germinate in germination media in 3-4 days. Explants were taken from plantlets, grown, and covered inside growing tubes.

Embryogenic culture 1: Callus formation was observed ten days after the establishment of culture in all explant types (Figure 1). A high amount of callus formation was achieved from both all explant types and all media. Only E1-5 medium containing 2 mg l^{-1} NAA with 89 % of callus formation rate was significantly different from other media and it produced a lower level of callus. The other media presented no important differences statistically in terms of callus formation performance (Table 2).

Table 2. Callus formation percentage from explant types and different medium and assessment of medium-explant interaction in embryogenic culture 1

Medium	Leaf	Petiole	Root collar disc	Cotyledon	Mean of medium
E1-1	95 (84)a	100 (90)a	100 (90)a	93.75 (81.1)a	97.18 (86.2)A
E1-2	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
E1-3	100 (90)a	100 (90)a	100 (90)a	93.75 (81.1)a	98.43 (87.7)A
E1-4	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
E1-5	100 (90)a	90 (81)a	100 (90)a	66 (57.49)b	89 (79.62)B
E1-6	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
E1-7	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
E1-8	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
Mean of explant	100 (90)A	98.12 (88.12)A	100 (90)A	94.18 (83.71)B	

LSD explant = 3.75, LSD medium =5.3, LSD explant x medium = 10.61, ($P<0.05$).

Embryogenic culture 2: In this experiment, calli induced embryogenic regeneration previously, however not transformed embryos were induced embryogenic regeneration again with different conditions. Likewise, in the first embryogenic culture, embryo formation was not observed, however, callus mass-producing continued.

Embryogenic culture 3: In the experiment, callus production was obtained from both explant types and all different media. Callus formation rates from different explants were significantly different. Leaf and cotyledon explants were the best explants types with the rate of 91.25% and 92.5%. They were followed by petiole and cotyledon stalk with a rate of 54.25% and 39% respectively (Table 3). It is found that there was no effect of glutamine statistically for callus production.

Differences between callus production means rate of media was significant statistically. Although E3-1 (1 mg l^{-1} NAA) was the best medium with 82.5% callus production rate, the least callus production was obtained from E3-4 (2 mg l^{-1} 2,4-D) medium with 63.5% (Table 3).

Interaction between medium and explant for callus formation was found significantly different. Leaf x E3-6 (2 mg l NAA), cotyledon x E3-6 (2 mg l^{-1} NAA) and cotyledon x E3-8 (2 mg l^{-1} 2,4-D) interactions were the best medium-explant combinations with 100%. The least callus production rate was found in cotyledon stalk x E3-6 (2 mg l^{-1} NAA), cotyledon stalk x E3-5 (1 mg l^{-1} NAA), and cotyledon stalk x E3-7 (1 mg l^{-1} 2,4-D) interactions with the rate of 26% (Table 3).

Embryo-like structures were formed in all of the embryogenic cultures (Figure 2). However, the growth of these structures was blocked and they could not grow more and germinated.

Table 3. Callus formation percentage from explant types and different medium and assessment of medium-explant interaction in embryogenic culture 3

Medium	Leaf	Petiole	Cotyledon	Cotyledon stalk	Mean of medium
E3-1	96 (82.62)abc	82 (67.84)bcde	96 (84.68)ab	56 (48.46)fgh	82.5 (70.90)A
E3-2	96 (82.62)abc	62 (52.19)efg	80 (72.00)bcd	32 (33.81)hıj	67.5 (60.16)BC
E3-3	94 (83.35)ab	50 (45.00)fgh	84 (72.00)bcd	44 (41.31)fghı	68 (60.41)BC
E3-4	78 (65.95)cde	44 (40.89)fghıj	96 (84.68)ab	34 (35.39)ghıj	63.5 (56.73)C
E3-5	94 (81.00)abc	56 (47.91)fgh	90 (75.68)abc	26 (27.77)ıj	66.5 (58.09)BC
E3-6	100 (90.00)a	44 (41.31)fghı	100 (90)a	26 (24.04)j	67.5 (61.34)BC
E3-7	92 (79.67)abc	42 (40.33)fghıj	94 (83.35)ab	26 (27.59)ıj	63.5 (57.74)BC
E3-8	80 (69.46)bcd	54 (47.30)fgh	100 (90.00)a	68 (55.58)def	75.5 (65.50)AB
Mean of explant	91.25 (79.33)A	54.25 (47.85)B	92.5 (81.55)A	39 (36.74)C	

LSD $_{explant}$ = 6.05, LSD $_{medium}$ = 8.56, LSD $_{explant\ x\ medium}$ = 17.12, (P<0.05).

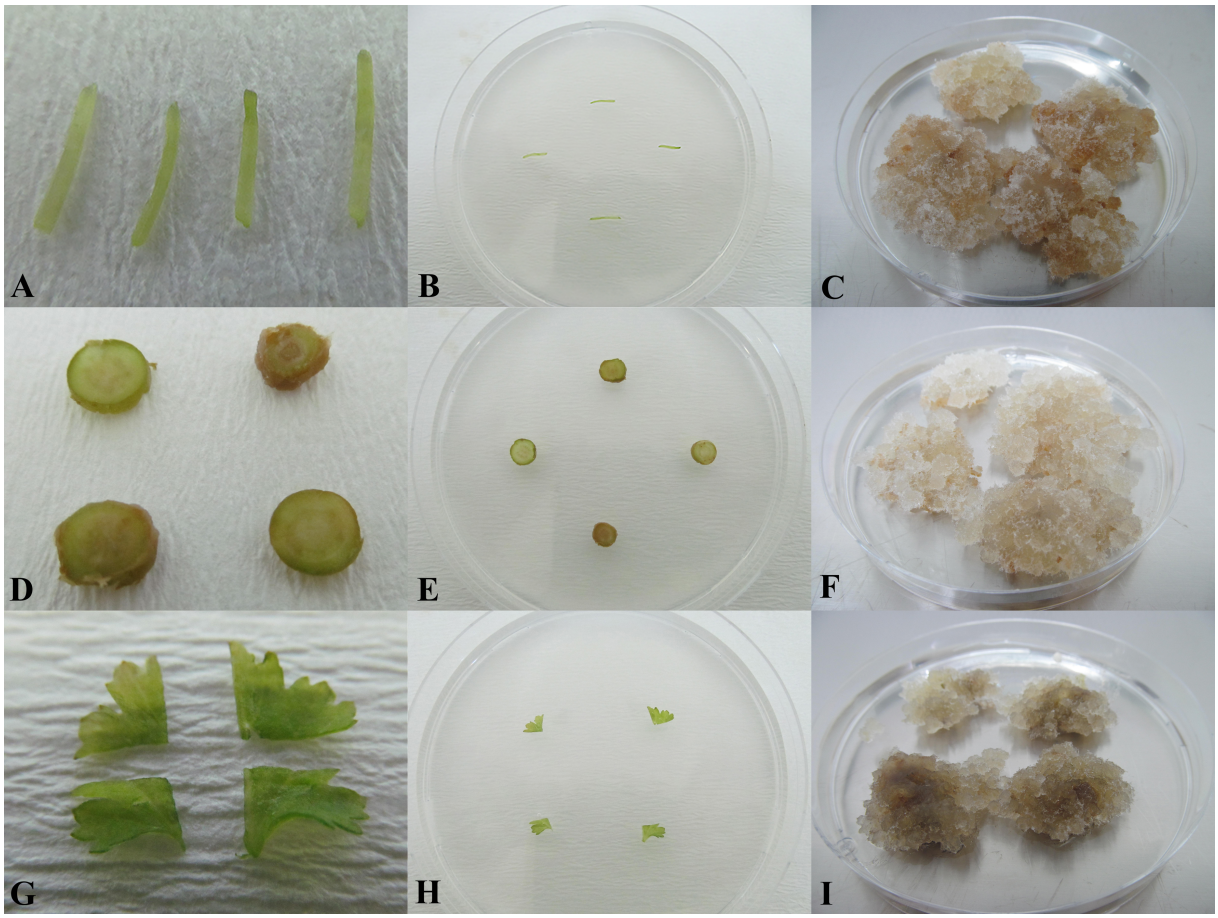

Figure 1. Explants and callus mass from explants A, B) Petiole, C) Callus from petiole, D, E) Root collar disc, F) Callus from root collar disc, G, H) Leaf, I) Callus from leaf.

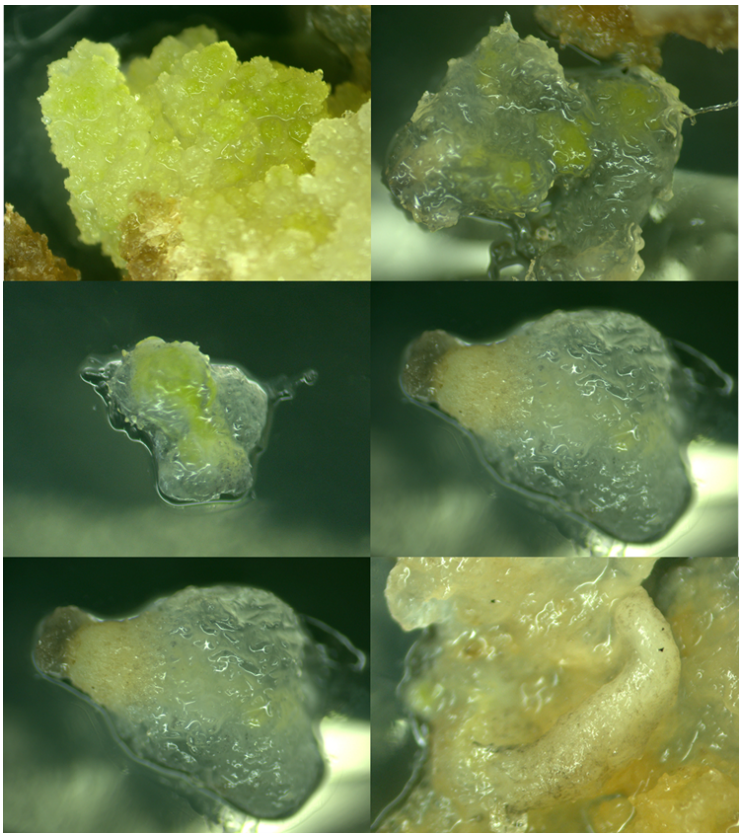

Figure 2. Callus formation and embryo-like structures in embryogenic cultures.

Organogenic culture: Callus formation was observed in a week. Some of the calluses with a color scale ranging from transparent-light color to brown in the first stages of culture showed green color formation with the effect of light in the later stages. According to both explant type and medium, callus formation rates were statistically important. The best callus formation rate observed in cultures from root collar discs with 93.75%. Cotyledon, leaf, and petiole followed it with the rate of 86.37%, 75%, and 71.25% respectively. The best callus formation obtained from O2 medium (1 mg l^{-1} 2,4-D + 2 mg l^{-1} BA) and O8 medium (1 mg l^{-1} 2,4-D + 2 mg l^{-1} 2iP) with the rate of 100%. The lowest callus yield was obtained from O3 medium (2 mg l^{-1} Kinetin) with 12.5%. Although most of the interactions between explant types and media gave a 100% callus formation rate, interactions of O3 (2 mg l^{-1} Kinetin) x leaf, O3 (mg l^{-1} Kinetin) x petiole, O3 (2 mg l^{-1} Kinetin) x cotyledon and O3 (mg l^{-1} 2iP) x leaf did not produce callus (Table 4).

Table 4. Callus formation percentage from explant types and different medium and assessment of medium-explant interaction in organogenic culture

Medium	Leaf	Petiole	Root collar disc	Cotyledon	Mean of medium
O1	100 (90)a	100 (90)a	100 (90)a	91 (76.6)bc	97.5 (86.65)AB
O2	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
O3	0 (0)	0 (0)	50 (45)d	0 (0)	12.5 (11.25)D
O4	100 (90)a	80 (69)c	100 (90)a	100 (90)a	95 (84.75)B
O5	100 (90)a	95 (84)ab	100 (90)a	100 (90)a	98.75 (88.5)AB
O6	0 (0)	5 (6)e	100 (90)a	100 (90)a	51.25 (46.5)C
O7	100 (90)a	90 (81)b	100 (90)a	100 (90)a	97.5 (87.75)AB
O8	100 (90)a	100 (90)a	100 (90)a	100 (90)a	100 (90)A
Mean of explant	75 (67.5)C	71.25 (63.75)D	93.75 (84.37)A	86.37 (77)B	

$LSD_{explant}$ = 2.98, LSD_{medium} = 4.63, $LSD_{explant\ x\ medium}$ = 8.43, ($P<0.05$).

4. Discussion

In this study, embryogenesis and organogenesis experiments of *Pelargonium quercetorum* Agnew were established for the first time and callus formation was achieved in all media and explant types used in all experiments.

Although *in vitro* studies have been reported in many *Pelargonium* species; *Pelargonium* × *Hortorum* L. H. Bailey (Vejsadová and Kuchtová-Jadrná 2008), *Pelargonium rapaceum* (L.) L'Hérit (Sukhumpinij et al., 2010), *Pelargonium graveolens* (Benazir et al., 2013), *Pelargonium radula* (Zuraida et al., 2014), *Pelargonium sidoides* DC (Kumar et al., 2015), there is no report *on Pelargonium quercetorum* Agnew. In the first experiment in this study, embryogenic cultures were established using combinations of 2,4-D and NAA as auxins and BA, Kinetin, and 2iP as cytokinins. Callus was obtained from all media. Brown and Charlwood (1986) also used 0.2 mg l^{-1} kinetin and 1 mg l^{-1} 2,4-D for callus production in their study on fragrant geranium species. Similarly, Benazir et al. (2013) obtained callus production in *P. graveolens* from an MS medium containing 20 µM NAA and 10 µM kinetin. However, in this study, callus production could not be obtained from media containing 10-60 µM 2,4-D.

The effect of glutamine on callus formation was found to be statistically insignificant. Similar results were reported by Amer et al. (2017). These researchers reported that glutamine had no effect on callus formation and shoot regeneration in two different varieties of rice plants. However, Pawar et al. (2015) reported that proline and glutamine had a positive effect on callus development in four different varieties of rice. These different results show that the effect of glutamine on callus formation *in vitro* may vary from species to species and even within species according to different varieties.

We observed that the best callus-forming medium was 1 mg l^{-1} NAA for *P. quercetorum* Agnew. In previous studies, it was determined that TDZ promoted callus formation for other *Pelargonium* species and varieties (Visser et al., 1992; Robichon et al., 1997). Qureshi and Saxena (1992) reported that regeneration type was determined by TDZ dose. Low concentrations (0.2 - 0.4 mg l^{-1}) stimulated adventitious shoot formation, while somatic embryos were observed at higher concentrations (2.2 mg l^{-1}). Sreedhar (1999) found that MS medium supplemented with various levels of 2,4-D was the primary medium for callus induction, however in contrast to reported studies in *P. graveolens*, the plant reacted poorly and showed no signs of growth. Sukhumpinij et al. (2010) found that the highest callus formation rate (100%) for *Pelargonium rapaceum* (L.) was observed in leaf explants cultured on media containing combinations of NAA and BAP, 2,4-D and BAP, and IAA and TDZ. In the presence of 2,4-D, none of the cultured explants formed shoots, and, in some cases, callus formed and then became necrotic and died. They reported that this was probably due to the toxic effects of auxin.

Agarwal and Ranu (2000) investigated *in vitro* plant regeneration potential of vegetatively propagated geraniums (*Pelargonium hortorum*). They found that combinations of zeatin and IAA or BA and IAA led to regeneration. They reported that zeatin and IAA combinations resulted in higher levels of regeneration and a number of regenerated shoots per explant compared to BA and IAA combinations.

Madden et al. (2005) examined modes of regeneration from hypocotyl explants with different cytokinin treatments [1 µM thidiazuron (TDZ), 4 µM TDZ or 8 µM N6-benzylaminopurine (BA), and 1 µM indole-3-acetic acid (IAA)] in *Pelargonium* x *hortorum* 'Scarlet Orbit' and three wild relatives *P. zonale*, *P. alchemilloides,* and *P. inquinans*. They found that *Pelargonium* x *hortorum* 'Scarlet Orbit' and *P. zonale* showed the same number of embryo-like structures in response to 1 µM TDZ. However, *P. alchemilloides* and *P. inquinans* showed weak embryogenic structures in response to all treatments. Similarly, in our study, embryo-like structures were formed in all embryogenic cultures, however, embryos did not grow.

Many studies have been conducted on different *Pelargonium* cultivars. Wojtania et al. (2004) compared the effects of meta-topoline and BA on *Pelargonium* × *hortorum* 'Bargpalais' proliferation. They found that regenerated shoots after meta-topolin treatment showed better quality and contained fewer abnormal shoots compared to shoots induced in the BA medium. Similar studies can be planned for *P. quercetorum* Agnew in the future.

Conclusion

We have established for the first time an effective callus production protocol for *Pelargonium quercetorum* Agnew. Preliminary studies to determine the appropriate conditions for somatic

embryogenesis have been successfully carried out. Based on this study, the development of new protocols can be aimed. The development and optimization of biotechnological approaches with the establishment of appropriate protocols for somatic embryogenesis will open some additional possibilities for further research in *Pelargonium quercetorum* Agnew. In the future, it is thought that important results will be obtained in areas such as the development of disease-resistant varieties, the application of techniques such as cell cultures, induced mutations and genetic transformation, and *in vitro* fertilization and embryo rescue studies.

Acknowledgements

This study was supported by Van Yuzuncu Yil University Scientific Research Projects Department with the project coded of FBA-2018-6282.

References

Agarwal, P. K., & Ranu, R. S. (2000). Regeneration of plantlets from leaf and petiole explants of *Pelargonium × hortorum*. *In Vitro Cellular and Developmental Biology - Plant*, *36*(5). https://doi.org/10.1007/s11627-000-0070-y

Agnew, A. D. Q. (1967). Contributions to the Flora of Iraq: IV: Notes on the Geraniaceae of Iraq, with a New Species of Pelargonium. *Kew Bulletin*, *21*(2). https://doi.org/10.2307/4108508

Altan, F., & Duru, M. (2017). Investigation of alkaloids in callus and multiple shoots obtained from *Catharantus roseus* L. *YYU J AGR SCI*, *27*(3), 337-346.

Amer, A. M., Mohamed, G. M., Hussein, M. H., Sedik, M. Z., & Aly, U. I. (2017). Effect of some of the natural organic sources on rice tissue culture. *Egyptian Pharmaceutical Journal*, *16*(3), 152. https://doi.org/10.4103/EPJ.EPJ_32_17

Bakhshaie, M., Babalar, M., Mirmasoumi, M., & Khalighi, A. (2010). Somatic embryogenesis and plant regeneration of *Lilium ledebourii* (Baker) Boiss., an endangered species. *Plant Cell, Tissue and Organ Culture*, *102*(2), 229–235. https://doi.org/10.1007/S11240-010-9726-4

Benazir, J. F., Suganthi, R., Chandrika, P., & Mathithumilan, B. (2013). *In vitro* regeneration and transformation studies on *Pelargonium graveolens* (geranium) - an important medicinal and aromatic plant. *Journal of Medicinal Plants Research*, *7*(38).

Benjamin, E. D., Ishaku, G. A., Peingurta, F. A., & Afolabi, A. S. (2019). Callus culture for the production of therapeutic compounds. *American Journal of Plant Biology*, *4*(4), 76-84. https://doi.org/10.11648/j.ajpb.20190404.14

Brown, J. T., & Charlwood, B. V. (1986). The accumulation of essential oils by tissue cultures of *Pelargonium fragrans* (Willd.). *FEBS Letters*, *204*(1). https://doi.org/10.1016/0014-5793(86)81397-7

Castillo, B., & Smith, M. A. L. (1997). Direct somatic embryogenesis from *Begonia gracilis* explants. *Plant Cell Reports*, *16*(6). https://doi.org/10.1007/s002990050244

Choffe, K. L., Victor, J. M. R., Murch, S. J., & Saxena, P. K. (2000). *In vitro* regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro Cellular and Developmental Biology - Plant*, *36*(1). https://doi.org/10.1007/s11627-000-0008-4

Daneshvar, M. H., Havil, M., & Lotfi Jalal-Abadi, A. (2022). Micropropagation of *Polianthes tuberosa* L. through direct organogenesis. *J. Plant Prod.*, *45*(2).

Davis, P.H. (1967). *Pelargonium* L. In: Davis PH (Ed.) Flora of Turkey and the East Aegean Islands. *Edinburgh University Press*, Edinburgh, 451–474.

Ebrahimzadeh, A., Fathollahzadeh, M., Aazami, M. A., & Hassanpouraghdam, M. B. (2022). *In vitro* direct and indirect regeneration of plants from nodal and petiole explants in *Pelargonium odoratissimum* (L.) Herit. *Acta Agriculturae Slovenica*, *118*(4). https://doi.org/10.14720/aas.2022.118.4.1723

Habiba, S. U., Ahasan, M. M., & Shimasaki, K. (2018). Effects of Ethrel on Organogenesis of Protocorm-like bodies in *Dendrobium kingianum In vitro*. *Plant Tissue Culture and Biotechnology*, *28*(1). https://doi.org/10.3329/ptcb.v28i1.37205

Kaval, I., Behçet, L., & Cakilcioglu, U. (2014). Ethnobotanical study on medicinal plants in Geçitli and its surrounding (Hakkari-Turkey). *Journal of Ethnopharmacology*, *155*(1). https://doi.org/10.1016/j.jep.2014.05.014

Kazeroonian, R., Mousavi, A., Kalate Jari, S., & Tohidfar, M. (2018). Factors Influencing *in Vitro* Organogenesis of *Chrysanthemum morifolium* cv. 'Resomee Splendid.' *Iranian Journal of Biotechnology*, *16*(2). https://doi.org/10.21859/ijb.1454

Kocak, M., Izgu, T., Sevindik, B., Tutuncu, M., Curuk, P., Simsek, O., Aka Kacar, Y., Teixeira Da Silva, J. A., & Mendi, Y.Y., (2014). Somatic embryogenesis of Turkish *Cyclamen persicum* Mill. *Scientia Horticulturae* 172, 26–33.

Kumar, V., Moyo, M., & Van Staden, J. (2015). Somatic embryogenesis of *Pelargonium sidoides* DC. *Plant Cell, Tissue and Organ Culture*, *121*(3), 571–577. https://doi.org/10.1007/S11240-015-0726-2/METRICS

Lü, J., Chen, R., Zhang, M., da Silva, J. A. T., & Ma, G. (2013). Plant regeneration via somatic embryogenesis and shoot organogenesis from immature cotyledons of *Camellia nitidissima* Chi. *Journal of Plant Physiology*, *170*(13). https://doi.org/10.1016/j.jplph.2013.03.019

Madden, J. I., Jones, C. S., & Auer, C. A. (2005). Modes of regeneration in *Pelargonium x hortorum* (Geraniaceae) and three closely related species. *In Vitro Cellular and Developmental Biology - Plant*, *41*(1). https://doi.org/10.1079/IVP2004605

Maślanka, M., & Bach, A. (2014). Induction of bulb organogenesis in *in vitro* cultures of tarda tulip (*Tulipa tarda* Stapf.) from seed-derived explants. *In Vitro Cellular and Developmental Biology - Plant*, *50*(6). https://doi.org/10.1007/s11627-014-9641-1

Mendi, Y. Y., Curuk, P., Kocaman, E., Unek, C., Eldogan, S., Gencel, G., & Çetiner, S. (2009). Regeneration of begonia plantlets by direct organogenesis. *African Journal of Biotechnology*, *8*(9).

Öktem, H., & Yücel, M. (2012). *Bitki Biyoteknolojisi ve Genetik: İlkeler. Teknikler ve Uygulamalar*. Ankara: Nobel Akademik Yayıncılık. p.113-134.

Pareek, A., & Kothari, S. L. (2003). Direct somatic embryogenesis and plant regeneration from leaf cultures of ornamental species of Dianthus. *Scientia Horticulturae*, *98*(4). https://doi.org/10.1016/S0304-4238(03)00078-5

Pawar, B., Kale, P., Bahurupe, J., Jadhav, A., Kale, A., & Pawar, S. (2015). Proline and glutamine improve *in vitro* callus induction and subsequent shooting in rice. *Rice Science*, *22*(6). https://doi.org/10.1016/j.rsci.2015.11.001

Qureshi, J. A., & Saxena, P. K. (1992). Adventitious shoot induction and somatic embryogenesis with intact seedlings of several hybrid seed geranium (*Pelargonium x hortorum* Bailey) varieties. *Plant Cell Reports*, *11*(9). https://doi.org/10.1007/BF00232687

Robichon, M. P., Renou, J. P., & Jalouzot, R. (1997). Plant regeneration of ivy leaved geranium through shoot organogenesis. *Plant Cell, Tissue and Organ Culture*, *49*(3). https://doi.org/10.1023/A:1005843901764

Röschenbleck, J., Albers, F., Müller, K., Weinl, S., & Kudla, J. (2014). Phylogenetics, character evolution and a subgeneric revision of the genus Pelargonium (Geraniaceae). *Phytotaxa*, *159*(2). https://doi.org/10.11646/phytotaxa.159.2.1

Rout, G. R., Mohapatra, A., & Jain, S. M. (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. In *Biotechnology Advances* (Vol. 24, Issue 6). https://doi.org/10.1016/j.biotechadv.2006.05.001

Saxena, G., Banerjee, S., Rahman, L., Mallavarapu, G. R., Sharma, S., & Kumar, S. (2000). An efficient *in vitro* procedure for micropropagation and generation of somaclones of rose scented Pelargonium. *Plant Science*, *155*(2). https://doi.org/10.1016/S0168-9452(00)00213-2

Sreedhar, D. (1999). Tissue culture of *Pelargonium graveolans* L.Her. Ex. A it (Geranium). *A Role of Biotechnology in Medicinal and Aromatic Plants*, *2*, 177–184.

Sukhumpinij, P., Kakihara, F., & Kato, M. (2010). *In vitro* regeneration from mature leaf explants of *Pelargonium rapaceum* (L.) L'Hérit. *Scientia Horticulturae*, *126*(3), 385–389. https://doi.org/10.1016/J.SCIENTA.2010.07.028

Taherpour, A., & Khojasteh, K. (2008). Comparison of the volatile constituents of *Pelargonium quercetorum* Agnew. of Iran with some of the other pelargonium species. *Asian Journal of Chemistry*, *20*(8), 6335.

Vejsadová, H., & Kuchtová-Jadrná, P. (2008). Indukce organogeneze u diploidních kultivarů *Pelargonium× hortorum* LH Bailey. *Acta Pruhoniciana*, *89*, 41–45.

Visser, C., Qureshi, J. A., Gill, R., & Saxena, P. K. (1992). Morphoregulatory role of thidiazuron: Substitution of auxin and cytokinin requirement for the induction of somatic embryogenesis in geranium hypocotyl cultures. *Plant Physiology*, *99*(4). https://doi.org/10.1104/pp.99.4.1704

Wojtania, A., Gabryszewska, E., & Marasek, A. (2004). Regeneration of *Pelargonium x hederaefolium* "Bonete" from petiole explants. *Acta Physiologiae Plantarum*, *26*(3), 255–262. https://doi.org/10.1007/S11738-004-0015-X/METRICS

Zhang, J., Gai, M. Z., Li, X. Y., Li, T. L., & Sun, H. M. (2016). Somatic embryogenesis and direct as well as indirect organogenesis in *Lilium pumilum* DC. Fisch., an endangered ornamental and medicinal plant. *Bioscience, Biotechnology and Biochemistry*, *80*(10). https://doi.org/10.1080/09168451.2016.1194178

Zuraida, A., Shukri, M., Sabrina, M., & Nazreena, O. (2015). Improvement of Regeneration of *Pelargonium radula* via Somatic Embryogenesis. *British Biotechnology Journal*, *5*(4). https://doi.org/10.9734/bbj/2015/15337