Biodiversity Journal, 2022,13 (4): 869–875 https://doi.org/10.31396/Biodiv.Jour.2022.13.4.869.875

Tissue culture techniques for callus induction in *Mentha rotundifolia* (L.) Huds and *M. pulegium* L. (Lamiales Lamiaceae)

Amira Benahmed*, Boualem Harfi, Imen Benchikhelhocine, Ibtissem Benbelkacem, Abla Daas & Khaoula Cherouana

[1]Biotechnology research center (CRBt.) Nouvelle ville, Ali Mendjeli 25000, Constantine, Algeria
*Corresponding author, e-mail: a.benahmed@crbt.dz

ABSTRACT Explants of two species of *Mentha rotundifolia* (L.) Huds and *Mentha pulegium* L. were evaluated for *in vitro* callus induction at Biotechnology Research Center (C.R.Bt), Algeria, during 2018, using MS medium supplemented with different hormonal combinations of NAA (naphthaleneacetic acid) and KIN (Kinetine) mg/l. Objective of our study is to develop a well-adopted technique for callus induction in these species by using MS medium with different concentrations of growth hormones. Internodes and leafs were used as explant source. Best response toward callus formation (100%) were observed for *M. pulegium* on the combinations M11 (0.1 mg/l NAA +0.5 mg/l KIN), M13 (1 mg/l NAA +0.5 mg/l KIN), M14 (1.5 mg/l NAA+0.5 mg/l KIN), M17 (0.5 mg/l NAA +1 mg/l KIN) and M19 (1.5 mg/l NAA+1 mg/l KIN). For the *M. rotundifolia*, the highest rate (68.75%) of callus formation was obtained on M6 (0.1 mg/l NAA+0.1 mg/l KIN). The best average callus surface of 84.68 mm^2 was obtained in M19 (1.5 mg/l NAA, 1 mg/l KIN) for *M. pulegium* and highest average callus surface (49.07 mm^2) was obtained in M7 (0.5 mg/l NAA, 0.1 mg/l KIN) for *M. rotundifolia*. Leafs explant induce important average callus surface than the internode explant for the both species of *Mentha* studied. The callus texture was friable and had two types of color: white and brown. The result demonstrated a well-adopted protocol for callus induction in these species of mentha from different explants source.

KEY WORDS Callus induction; *Mentha rotundifolia* (L.) Huds; *Mentha pulegium* L.

Received 02.10.2022; accepted 24.11.2022; published online 30.12.2022

INTRODUCTION

Medicinal plants have long been used for health care purpose throughout the world since ancient times. Many of medicinal plants have limited natural habitats and only grow in certain geographical and environmental conditions.

The *in vitro* propagated medicinal plants furnish a ready source of uniform, sterile, and compatible plant material for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Anis & Faisal, 2005; Uppeandra et al., 2005). Further, genetic improvement is another approach to augment the drug yielding capacity of the plant (Benahmed et al., 2019; Karuppusamy et al., 2006).

The variation generated by use of tissue culture regeneration techniques has resulted in improvement of diverse commercial crops (Reisch, 1983).

Somaclonal variations commonly appear after tissue culture, which involve a callus stage (Larkin & Scowcraft, 1981). Callus is undifferentiated mass of rapidly proliferating cells, can be obtained by culturing explants source (seed, node, bud, leaves, meristem and root tips etc) on nutrient medium containing specific growth regulators along with a standard recipe of chemicals.

According to Van Eck et al. (1999), plant regeneration from callus cultures of mint depended on explant source, genotype, and culture medium components. The objective of this research was to establish protocols for callus induction of *Mentha rotundifolia* (L.) Huds and *M. pulegium* L. (Lamiales Lamiaceae).

MATERIAL AND METHODS

Callus induction

Callus induction was performed from vitroplant of *Mentha rotundifolia* and *M. pulegium* obtained according to the protocol recommended by Benahmed et al. (2018) and Benahmed et al. (2019). Vitroplants of 4 weeks were cut under aseptic conditions into leafs fragments and internods fragments of about 0.5 cm. These were cultured at the rate of 4 explants per culture pot containing 50 mL of MS Medium with different hormonal combinations NAA (0; 0.1; 0.5; 1; 1.5) and KIN (0; 0.1; 0.5; 1) mg/l.

Culture conditions

MS culture media were supplemented with 0.8% agar, 3% sucrose and pH adjusted to 5.6–5.8 before autoclaved at 121 °C during 20 min. All the cultures were incubated in a growth chamber at 23 ± 1 °C and total darkness during 45 days.

Callus transfer

After callus induction from the explants (two months of culture), the calli were transferred into the fresh medium after every two weeks for further proliferation and maintenance.

Brown, watery and dead calli were removed during each subculture. Friable, having green roundish callus were selected.

Frequency of callus induction was calculated using the following formula: Frequency of callus induction (%) = Number of explants induced callus× 100 / Total number of explants inoculated.

Statistical analysis

Each experiment repeated three times and the results presented as mean ± standard error meaning (SEM.). After data normality tests, the statistical analysis was performed. It carried out with XLSTAT software according to variance analysis (one way ANOVA) and Tukey's test for the obtained results (< 0.05).

RESULTS AND DISCUSSION

Callus reactivity

The reactivity of the explants, on the MS culture medium with the different hormonal combinaisons tested, was manifested from the first week (7th day) of cultivation and was reflected in the ends of the rods and in the injuries given to the leaf explant. The first step is the swelling of the explants, then cell division followed by significant and uncontrolled proliferation of its cells completely covering it with the implant which will form a callus.

Callus development

Several hormonal combinations have been tested to determine which ones allow to achieve the highest percentage of callogenesis (Table 1). All hormonal combinations used induced reactivity of the explants placed in culture. However, differences were recorded in relation to the response time and the rate of callogenesis after two months of culture (Table 1). Indeed, For the species *M. pulegium*, the hormonal combinations M11 (0.1mg/l NAA+0.5 mg/l KIN), M13 (1 mg/l NAA+0.5 mg/l KIN) , M14 (1.5 mg/l NAA+0.5 mg/l KIN), M15 (0 mg/l NAA+1 mg/l KIN), M16 (0.1 mg/l NAA+1 mg/l KIN) and M17 (0.5 mg/l NAA+1 mg/l KIN) were the fastest and the first to show callogenesis. The results showed that the maximum rates (100%) of callus formation were recorded on the combinations M11 (0.1 mg/l NAA+0.5 mg/l KIN), M13 (1 mg/l NAA+0.5 mg/l KIN), M14 (1.5 mg/l NAA+0.5

mg/l KIN), M17 (0.5 mg/l NAA+1 mg/l KIN) and M19 (1.5 mg/l NAA+1 mg/l KIN). These calli were white or brownish in colour and friable in nature (table 1).

Thus high rates between (80–94%) were recorded on the combinations M4 (1.5 mg/l NAA+0 mg/l KIN), M7 (0.5 mg/l NAA+0.1 mg/ l KIN), M8 (1 mg/l NAA+0.1 mg/l KIN), M9 (1.5 mg/l NAA+0.1 mg/l KIN), M16 (0.1 mg/l NAA+1 mg/l KIN) and M18 (1 mg/l NAA+1 mg/l KIN). Low callus formation rates between (12–63%) were obtained for combinations M1 (0.1 mg/l NAA+0 mg/l KIN), M3 (1 mg/l NAA+0 mg/l KIN), M5 (0 mg/l NAA+0.1 mg/l KIN), M6 (0.1 mg/l NAA+0.1 mg/l KIN), M10 (0 mg/l NAA+0.5 mg/l KIN) and M15 (0 mg/l NAA+1 mg/l KIN). A complete absence of callus formation (0%) was recorded on the combinations M0 (0 mg/l NAA+0 mg/l KIN), M2 (0.5 mg/l NAA+0 mg/l KIN) and M12 (0.5 mg/l NAA+0.5 mg/l KIN).

For the *M. rotundifolia* species, the highest rate (68.75%) of callus formation was obtained on M6 (0.1 mg/l NAA+0.1 mg/l KIN) followed by M4 (1.5 mg/l NAA+0 mg/l KIN) with a rate of 43.75%. Low rates of callus formation (6–25%) were recorded on M1 (0.1 mg/l NAA+0 mg/l KIN), M2 (0.5 mg/l NAA+0 mg/l KIN), M3 (1 mg/l NAA+0 mg/l KIN), M5 (0 mg/l NAA+0.1 mg/l KIN), M7 (0.5 mg/l NAA+0.1 mg/l KIN), M8 (1 mg/l NAA+0.1 mg/l KIN), M9 (1.5 mg/l NAA+0.1 mg/l KIN), M17 (0.5 mg/l NAA+1 mg/l KIN), M18 (1 mg/l NAA+1 mg/l KIN) and M19 (1.5 mg/l NAA+1 mg/l KIN). All obtained callus were white in colour and friable in nature (Table1). Total absence of callus formation (0%) was recorded on the combinations: M0 (0 mg/l NAA+0 mg/l KIN), M10 (0 mg/l NAA+0.5 mg/l KIN), M11 (0.1 mg/l NAA+0.5 mg/l KIN), M12 (0.5 mg/l NAA+0.5 mg/l KIN), M13 (1 mg/l NAA+0.5 mg/l KIN) , M14 (1.5 mg/l NAA+0.5 mg/l KIN), M15 (0 mg/l NAA+1mg/l KIN) and M16 (0.1 mg/l NAA+1mg/l KIN).

In order to study the effect of explants type on callus formation, two explants (enternodes and leaves) from *M. pulegium* and *M. rotundifolia vitroplants* were cultured on MS medium with different hormonal combinaisions.

Enternodes explant induce more callus formation than leaf explants for the both species of Mentha studied where enternodes explants of *M. pulegium* showed 63.13% callus formation while 62.19% leaf explants produced calli. In the same, for *M. rotundifolia*, enternodes explants showed 17% callus formation while 10% leaf explants produced calli.

Genotype played a significant role in callus induction in cultures. *M. pulegium* explants response was significantly better compared to *M. rotundifolia* explants - enternodes and leaves of *M. pulegium* recorded callus induction with a rate 62-64% in the different plant growth regulators supplemented in MS media. *Mentha pulegium* explants was observed to have better callus formation compared to *M. rotundifolia* explants across all treatments. The explanation to this could be attributed to genealogy influences.

Callus surface

The average callus surface was measured after two months of culture, analysis of the results (Table 1) shows that the average callus surfaces vary according to the different hormonal combinations, the explant type and genotype.

Different hormonal combinations in MS medium have been tested in order to determine the best callus surface. Indeed, For *M. pulegium*, an average callus surface between (50–85 mm^2) was obtained on MS medium with the hormonal combinaisons M4 (1.5 mg/l NAA+0 mg/l KIN), M8 (1 mg/l NAA+0.1 mg/l KIN), M10 (0 mg/l NAA+0.5 mg/l KIN), M11 (0.1 mg/l NAA+0.5 mg/l KIN), M13 (1mg/l NAA+0.5 mg/l KIN), M14 (1.5 mg/l NAA+0.5 mg/l KIN), M16 (0.1 mg/l NAA+1 mg/l KIN), M17 (0.5 mg/l NAA+1 mg/l KIN), M18 (1 mg/l NAA+1 mg/l KIN) and M19 (1.5 mg/l NAA+1 mg/l KIN), with the maximun average callus surface of 84.68 mm^2 in M19 (1.5 mg/l NAA, 1 mg/l KIN). Average callus surface between (20–40 mm^2) were obtained in hormonal combination M1 (0.1 mg/l NAA+0 mg/l KIN), M3 (1 mg/l NAA+0 mg/l KIN), M5 (0 mg/l NAA+0.1 mg/l KIN), M6 (0.1 mg/l NAA+0.1 mg/l KIN), M7 (0.5 mg/l NAA+0.1 mg/l KIN), M9 (1.5 mg/l NAA+0.1 mg/l KIN) and M15 (0 mg/l NAA+1 mg/l KIN). The lowest average callus surface (0mm2) was obtained on the combinations M0, M2 (0.5 mg/l NAA+0 mg/l KIN) and M12 (0.5 mg/l NAA+0.5 mg/l KIN) (Table 1).

For *M. rotundifolia*, the highest average callus surface (49.07 mm^2) was obtained in M7 (0.5 mg/l

NAA + 0.1mg/l KIN). Hormonal combinaitions M5 (0 mg/l NAA+0.1 mg/l KIN), M6 (0.1 mg/l NAA+0.1 mg/l KIN), M8 (1 mg/l NAA+0.1 mg/l KIN) and M9 (1.5 mg/l NAA+0.1 mg/l KIN) caused callus formation with an average surface between (20–33 mm^2) and less than 10 mm^2 obtained in M1 (0.1 mg/l NAA+0 mg/l KIN), M2 (0.5 mg/l NAA+0 mg/l KIN), M4 (1.5 mg/l NAA+0 mg/l KIN), M17 (0.5 mg/l NAA+1 mg/l KIN), M18 (1 mg/l NAA+1 mg/l KIN) and M19 (1.5 mg/l NAA+1 mg/l KIN). The lowest average callus surface (0 mm^2) was obtained on the combinations M0, M10 (0 mg/l NAA+0.5 mg/l KIN), M11 (0.1 mg/l NAA+0. 5mg/l KIN), M12 (0.5 mg/l NAA+0.5 mg/l KIN), M13 (1 mg/l NAA+0.5 mg/l KIN), M14 (1.5 mg/l NAA+0.5 mg/l KIN), M15 (0 mg/l NAA+1 mg/l KIN) and M16 (0.1 mg/l NAA+1 mg/l KIN) (Table 1).

Explant type affect significantly the average callus surface in *M. pulegium.* Leaf explant induce the important average callus surface than the enternodes explant where leaf explants of *M. pulegium* showed an average callus surface of 56.34 mm^2 instead of 36.20 mm^2 obtained by enternodes explants. For *M. rotundifolia*, there is no significant differences among explant types for callus surface, leaf explants showed 14.45 mm^2 of average callus surface instead of 7.87 mm^2 obtained by enternodes explants.

A significant effect of genotype was observed in the average of callus surface. *M. pulegium* explants callus surface was significantly important compared to *M. rotundifolia* explants in the different plant growth regulators supplemented in MS media. *Mentha pulegium* explants was observed to have better average callus surface (46.27 mm^2) compared to *M. rotundifolia* explants (11.16 mm^2) across all treatments.

DISCUSSION

Callus induction is the best way to create somaclonal variations in crop plants. The main objective of our study is to know the best hormonal combinations to induce callogenesis for the two mint species studied. The potential of the two mint species for callus induction was observed significantly different on MS medium, response of *M. pulegium* for callus induction was significantly better compared to *M. rotundifolia*. It may be related by the interaction between endogenous phytohormones of the explants of species and growth regulators absorbed from the culture medium, which will influence the callus development (Asnawati et al., 2002).

Our results showed variations among each treatment (hormonal combinations). For *M. Pulegium,* callus induction rate (100%) was recorded on treatments MS+ (0.1 mg/l NAA+0.5 mg/l KIN), (1 mg/l NAA+0.5 mg/l KIN), (1.5 mg/l NAA+0.5 mg/l KIN), (0.5 mg/l NAA+1 mg/l KIN) and (1.5 mg/l NAA+1 mg/l KIN). For *M. rotundifolia* the best callus induction rate (68.75%) was obtained on M6 (0.1 mg/l NAA+0.1 mg/l KIN). In KIN and NAA combinations, various type of calli occurred. These calli were both white and brownish in colour and friable in nature. The white callus in an embryogenic tissue indicates that the callus state is relatively good (Ariati, 2012). In our result, brown calli were obtained mostly on MS medium with high concentration of NAA witch is in correlation with result reported by Al-Mayahi et al. (2018) and Khater et al. (2021) where the calli became brown in the medium containing high auxin content.

In vitro, callus induction depended on both medium and PGRs combination. Callus induction must be dependent on the genetic potentials of the variety and secondly the combination of hormones at different concentration. Callogenesis abilities depend on many parameters, and traditional methods of plant regeneration from callus involve the manipulations of the auxins to cytokinins balance. Role of auxin alone or in combination with cytoknin for callus proliferation is well documented (Hammerschlag et al., 1985; Jain et al., 1988; Niedz et al., 1989; Verhagen & Wann, 1989; Roy & De, 1990).

In our study, for the both species, leaf cultures responded better than nodal cuttings culture in terms of surface cultures producing callus. Similar results were reported by Thomas & Maseena, 2006 on *Cardiospermum halicacabum* L. (Sapindaceae) In nodal cuttings, calli were formed from the basal cut end whereas in leaf cultures the calli were produced all over the surface of the leaf. Darvishi et al. (2014) reported that leaf explant of Pennyroyal showed the highest effects on callus diameter in first and second measurements. Also, according to Islam & Alam (2018), leaf disc explants showed better

Type of explants	Culture media	Growth regulator (mg/l)		*Mentha pulegium*				*Mentha rotundifolia*			
		ANA	KIN	Callus Reactivity	% callogenesis	Callus morphology	Mean callus surface (mm 2)	Callus Reactivity	% callogenesis	Callus morphology	Mean callus surface (mm 2)
Inter-node explants	M_0	0	0	-	0	-	0.00 e	-	0	-	0.00c
	M_1	0.1	0	+	25	brownish, friable	31.60± 22.04 abcde	+	12.5	whitish, friable	11.71±7.74bc
	M_2	0.5	0	+	0	-	0.00 e	+	87.5	whitish, friable	20.15±10.21bc
	M_3	1	0	++	37.5	brownish, friable	16.32±12.40 cd	++	37.5	whitish, friable	35.20±23.12bc
	M_4	1.5	0	+++	75	brownish, friable	45.74±38.77 abcde	+	25	whitish, friable	18.42±16.55bc
	M_5	0	0.1	+++	37.5	whitish, friable	15.95±11.19 cd	-	0	-	0.00c
	M_6	0.1	0.1	+++	87.5	brownish, friable	23.70±5.90 bcd	++	100	whitish, friable	21.91±12.31bc
	M_7	0.5	0.1	+++	87.5	whitish, friable	39.78±18.35 abcde	-	0	-	0.00c
	M_8	1	0.1	+++	75	whitish, friable	39.08±13.77 abcde	+	0	-	0.00c
	M_9	1.5	0.1	+++	87.5	whitish, friable	36.08±12.63 abcde	+	12.5	whitish, friable	0.00c
	M_{10}	0	0.5	+	25	whitish, friable	30.57±6.24 abcde	++	0	-	0.00c
	M_{11}	0.1	0.5	+++	100	whitish, friable	70.38±38.37 abcde	+	0	-	0.00c
	M_{12}	0.5	0.5	+	0	-	0.00 e	-	0	-	0.00c
	M_{13}	1	0.5	+++	100	whitish, friable	47.10±2.62 abcde	+	0	-	0.00c
	M_{14}	1.5	0.5	+++	100	whitish, friable	41.38±21.73 abcde	-	0	-	0.00c
	M_{15}	0	1	+	37.5	brownish, friable	14.83±5.92 d	-	0	-	0.00c
	M_{16}	0.1	1	+++	100	brownish, friable	66.45±17.44 abcde	+	0	-	0.00c
	M_{17}	0.5	1	+++	100	whitish, friable	56.28±29.00 abcde	+	25	whitish, friable	19.39±14.40bc
	M_{18}	1	1	+++	87.5	whitish, friable	72.23±30.12 abcde	+	12.5	whitish, friable	10.94±10.86bc
	M_{19}	1.5	1	+++	100	whitish, friable	73.18±34.19 abcde	+	37.5	whitish, friable	19.65±4.82bc
Leaves explants	M_0	0	0	-	0	-	0.00 e	-	0	-	0.00c
	M_1	0.1	0	+	0	-	0.00 e	+	0	-	0.00c
	M_2	0.5	0	+	0	-	0.00 e	-	0	-	0.00c
	M_3	1	0	+++	31.25	brownish, friable	53.57±13.74 abcde	++	0	-	0.00c
	M_4	1.5	0	+++	100	brownish, friable	88.17±19.35 abcd	++	0	-	0.00c
	M_5	0	0.1	-	0	whitish, friable	38.82±25.10 abcde	+	50	whitish, friable	65.45±57.39 ab
	M_6	0.1	0.1	-	37.5	brownish, friable	37.66±24.13 abcde	++	37.5	whitish, friable	31.96±8.06bc
	M_7	0.5	0.1	++	87.5	brownish, friable	40.84±13.11 abcde	++	50	whitish, friable	98.14±50.79a
	M_8	1	0.1	+++	100	brownish, friable	77.83±35.04 abcde	++	37.5	whitish, friable	43.76±6.30ab
	M_9	1.5	0.1	+++	100	whitish, friable	38.01±14.28 abcde	+	25	whitish, friable	49.61±43.21ab
	M_{10}	0	0.5	+	50	whitish, friable	104.71±39.56 ab	+	0	-	0.00c
	M_{11}	0.1	0.5	+++	100	brownish, friable	69.76±15.03 abcde	+	0	-	0.00c
	M_{12}	0.5	0.5	-	0	-	0.00 e	-	0	-	0.00c
	M_{13}	1	0.5	+++	100	whitish, friable	116.60±49.77 a	-	0	-	0.00c
	M_{14}	1.5	0.5	+++	100	whitish, friable	111.47±70.26 a	-	0	-	0.00c
	M_{15}	0	1	+++	50	whitish, friable	45.97±27.26 abcde	-	0	-	0.00c
	M_{16}	0.1	1	+++	87.5	brownish, friable	48.66±19.16 abcde	+	0	-	0.00c
	M_{17}	0.5	1	+++	100	whitish, friable	102.47±50.46 abc	+	0	-	0.00c
	M_{18}	1	1	++	100	brownish, friable	56.08±27.49 abcde	-	0	-	10.94±10.86bc
	M_{19}	1.5	1	+++	100	whitish, friable	96.17±47.90 abcd	+	0	-	19.65±4.82bc

Table 1. Effect of growth regulators on callus reactivity, percentage of callogenesis, callus morphology and mean callus surface of *Mentha rotundifolia* and *M. pulegium* from enternode and leaf fragments.

performance as explant source than internodal segments for callus induction. According to Passey et al. (2003), in strawberry (*Fragaria* x *ananassa* Duch.), of the various explants (leaf disc, petiole, stipule and root) used, leaf-derived calli gave the maximum callus induction and regeneration response.

CONCLUSIONS

An efficient and reproducible protocol for high frequency callus formation from leaf and nodal cuttings explant in *M. pulegium* and *M. rotundifolia* has been **standardized. A maximum rates** (100%) of callus formation and an average callus surface of 84.68 mm^2 were recorded in *M. pulegium* on MS medium suplemented with 1.5 mg/l NAA+1 mg/l KIN (M19). For the *M. rotundifolia* species, the highest rate (68.75%) of callus formation and an average callus surface of 26.93 mm^2 were obtained on MS medium suplemented with 0.1 mg/l NAA+0.1 mg/l KIN (M6). Such a high callogenesis frequency is always useful for the large-scale production of somaclonal varieties of these species. This method have more potentials and suitable for further genetic studies.

REFERENCES

Al-Mayahi A.M.W., Ali A.H. & Shareef H.J., 2018. Influence of cold pretreatment on shoot regeneration from callus in date palm (*Phoenix dactylifera* L.) cv.'Barhee'. Journal of Genetic Engineering and Biotechnology, 16: 607–612. https://doi.org/10.1016/j.jgeb.2018.07.002

Anis M. & Faisal M., 2005. In vitro regeneration and mass multiplication of *Psoralea corylifolia* - An endangered medicinal plant. Indian Journal of Biotechnology, 4: 261–264.

Asnawati-Wattimena G.A., Machmud M. & Purwito A., 2002. Study of regeneration and production of mesophyll protoplast leaves of several potato plant clones (*Solanum tuberosum* L.). Bulletin Agronomy, 30: 87–91.

Benahmed A., Harfi B., Benbelkacem I., Daas A., Laouer H., Belkhiri A., 2018. *In vitro* propagated *Mentha rotundifolia* (L.) Huds and antioxidant activity of its essential oil. Current Issues in Pharmacy and Medical Sciences, 31: 204–208. https://doi.org/10.1515/cipms-2018-0038

Benahmed A., Harfi B. and Belkhiri A., 2019. Biological activity of essential oils of *Mentha pulegium* from field-grown and acclimated *in vitro* plants. Current Science, 116: 1897–1904.

Darvishi E., Kazemi E., Kahrizi D., Bahraminejad S., Mansouri M., Chaghakaboudi S.R. & Khani Y., 2014. Optimization of Callus Induction in Pennyroyal (*Mentha pulegium*). Journal of Applied Biotechnology Reports, 1*:* 97–100.

Hammerschlag F.A., Bauchan G. & Scorza R., 1985. Regeneration of peach plants from callus derived from immature embryos. Theoretical and Applied Genetics, 70: 248–251.

Islam A.T.M.R. & Alam M.F., 2018. *In vitro* callus induction and indirect organogenesis of *Mentha piperita* (L.) - An aromatic medicinal plant. GSC Biological and Pharmaceutical Sciences, 4: 49–60. https://doi.org/10.30574/gscbps.2018.4.3.0078

Jain R.K., Choudhury J.B., Sharma D.R. & Friedt W., 1988. Genotypic and media effects on plant regeneration form cotyledon explants cultures of some Brassica species. Plant Cell, Tissue and Organ Culture, 14: 197–206.

Larkin P.J. & W.R. Scowcraft, 1981. Somaclonal variation a novel source of variability from cell cultures of plant improvement. Theoretical and Applied Genetics, 60: 197–214.

Khater N., Benahmed A., Zereg N. & Cherouana K., 2021.Callogenesis induction of *Ilex aquifolium* L. (Aquifoliales Aquifoliaceae). Biodiversity Journal, 12: 265–272. https://doi.org/10.31396/Biodiv.Jour.2021.12.1.265.272

Karuppusamy S., Kiranmai C., Aruna V. & Pullaiah T., 2006. Micropropagation of *Vanasushava pedata* - An Endangered medicinal plant of South India. Plant Tissue Culture and Biotechnology, 16: 85–94. https://doi.org/10.3329/ptcb.v16i2.1109

Niedz R.P., Smith S. S., Dunbar K.S., Stephens C.T. & Murakishi H.H., 1989. Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo*. Plant Cell, Tissue and Organ Culture, 18: 313–319.

Passey A.J., Barrett K.J. & James D.J., 2003. Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria* x *ananassa* Duch.) using a range of explant types. Plant Cell Reports, 21: 397–401. https://doi.org/10.1007/s00299-002-0530-4

Reisch B., 1983. Genetic variability in regenerated plants, pp. 748–769. In: Evans D.A., Sharp W.R., Ammirato P.V. and Yamada Y. (Eds.), Handbook of plant cell culture. Vol. 1. Techniques for propagation and breeding. Macmillan, New York.

Roy A.T. & De D.N., 1990. Tissue Culture and plat regeneration from immature embry explants of *Calotropis gigantea* Linn. R. Br. Plant Cell, Tissue and Organ Culture, 20: 229–233.

Thomas T.D. & Maseena E.A., 2006. Callus induction and plant regeneration in *Cardiospermum halicacabum* Linn. - An important medicinal plant. Scientia Horticulturae, 108: 332–336.

Uppenadra D., Sumit M. & Meena J., 2005. Organogenesis, embryogenesis, and synthetic seed production in *Arnebia euchroma* - a critically endangered medicinal plant of the Himalaya. In Vitro Cellular & Developmental Biology - Plant, 41: 244–248.

Van Eck J.M. & Kitto S.L., 1990. Callus Initiation and Regeneration in *Mentha*. HortScience, 25: 804–806.

Verhagen S.A. & Wann S.R., 1989. Norway spruce somatic embryogenesis: high frequency initiation from light-cultured mature embryos. Plant Cell, Tissue and Organ Culture, 16: 100–111.