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ORIGINAL PAPER

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DEVELOPMENT OF AN EFFICIENT *in vitro* CALLUS PROLIFERATION PROTOCOL FOR EDIBLE WILD RHUBARB (*Rheum ribes* L.)

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ABSTRACT

Rheum ribes L. is a perennial wild species. Young shoots and flower bunches are freshly consumed, and root and rhizomes are generally used for medicinal purposes. The aim of the present study was to improve the callus proliferation protocol for *R. ribes* L. under *in vitro* conditions. For callus induction, hypocotyl explants taken from 14-day old plantlets germinated in Murashige and Skoog (MS) media were cultured in MS media with 9 plant growth regulator (PGR) combinations containing 6-benzylaminopurine (BAP) (2, 3, and 4 mg/L) + naphthylacetic acid (NAA) (0.1, 0.5, and 1 mg/L). Then, for callus proliferation, 4 PGR combinations containing NAA (0.2 mg/L) + thidiazuron (TDZ) (0.5, 1, 2, and 3 mg) were used in the first set of experiments, and 36 PGR combinations containing BAP (1, 2, 3, and 4 mg/L) + indole-3-butyric acid (IBA) (0.2, 0.5, and 1 mg/L), BAP (1, 2, 3, and 4 mg/L) + NAA (0.2, 0.5, and 1 mg/L), and TDZ (1, 2, 3, and 4 mg/L) + NAA (0.2, 0.5, and 1 mg/L) were used in the second set of experiments. At the end of the second set of experiments, the greatest callus regeneration ratios were obtained due to the combinations including BAP and IBA as well as the low-dose TDZ- (especially 1 mg/L) and NAA- (0.2, 0.5, 1 mg/L) combinations. Regarding callus fresh weights, TDZ + NAA combinations were found to be more successful. The greatest callus fresh weight (12.7 ±0.4 g) was obtained from MS medium supplemented with 2 mg/L TDZ and 0.2 mg/L NAA.

Key words: calli, explant, multiplication, plant growth regulator, Polygonaceae, *R. ribes* L.

INTRODUCTION

Rheum ribes L., belonging to the *Rheum* genus of the Polygonaceae family, is a perennial wild species. There are about 60 species in this genus [Anjen et al. 2003]. *Rheum ribes* L. naturally grows in stony and sloped sites of the Eastern Anatolia region of Turkey at altitudes between 1800–2800 m. It also has a natural spread in Northern Iraq, West and Northwestern Iran, Pakistan, Afghanistan, Lebanon, and the Eastern Anatolia region of Turkey [Cullen 1967]. Due to their nutritional attributes, young shoot and flower clusters of *R. ribes* L. are freshly consumed in the Eastern Anatolia region of Turkey. Particularly, the roots and rhizomes are known to have significant medicinal attributes. *Rheum* species are used in the treatment of hemorrhoids [Çakılcıoğlu and Türkoğlu 2009], and inflammations [Hu et al. 2014]. These species have also antiallergic [Matsuda et al. 2004], antiviral [Chang et al. 2014], antibacterial [Alaadin et al. 2007], and anti-cancerous [Rajkumar et. al. 2011] characteristics.

Tissue culture techniques allow mass propagation of various plants, endangered, endemic, and wild species as well, the preservation of germplasm, and facilitate the production of secondary metabolites. *In vitro* techniques have been used for the micro propagation of *Rheum* species, including *R. ribes* [Farzami Sepehr and Ghorbanli 2002, Farzami Sepehr and Ghorbanli 2005, Tuncer and Günsan 2017], *R. emodi* [Malik et al. 2010, Tabin et al. 2018], *R. coreanum* [Mun

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and Mun 2016], *R. webbianum* [Rashid et al. 2014], *R. spiciforme* [Tabin et al. 2016], *R. franzenbachii* [Wang et al. 2011], *R. rhaponticum* [Kozak and Sałata 2011], and *R. rhabarbarum* [Clapa et. al. 2020]. Callus and shoot regeneration have been achieved in different rates based on the species and culture medium. Due to the medicinal importance of *Rheum* species, economically valuable secondary metabolites are used in the food and pharmaceutical industries. For example, flavors and pharmaceuticals are produced under controlled laboratory conditions via *in vitro* callus cultures [Farzami Sepehr and Ghorbanli 2002, Farzami Sepehr and Ghorbanli 2005, Wang et al. 2011, Mun and Mun 2016]. The main objective of the present study was to develop an efficient *in vitro* callus proliferation protocol for *Rheum ribes* L. Besides, callus regeneration capacity in MS media [Murashige and Skoog 1962] with different PGR combinations and, *in vitro* shoot regeneration capacities of these PGR combinations were also investigated.

MATERIALS AND METHODS

Plant material and seed sterilization. Wild rhubarb (*Rheum ribes* L.) seeds were obtained from the Gürpınar town of the Van province (38°8'20.75"N, 43°30'55.15"E, 1730 m) situated in the Eastern Anatolia region of Turkey. The seeds were kept in a 0.3% benomyl solution for an hour to remove fungal disease agents and then washed thoroughly with distilled water for an hour. The seeds were further manipulated in a laminar flow cabin to prevent any possible bacterial infections, kept in 70% ethyl alcohol for 10 s, and washed then with bidistilled water. Following this process, the seeds were kept in a 50% sodium hypochlorite solution with 1–2 drops of Tween-20 for 10 min and washed with bidistilled water for 3 min three times. Sterilized seeds were placed on filter papers in to remove any excess water.

***In vitro* germination.** The sterilized seeds were sown into MS culture media supplemented with 30 g/L sucrose, 200 mg/L GA_3, and 50 mg/L citric acid; 8 mL aliquots of MS medium were dispensed into 60 × 15 mm sterile Petri dishes (5 seed/Petri dish). Seeds were sown in a total of 50 Petri dishes. Seeded Petri dishes were sealed with parafilm and kept at 4°C for about 25–30 days under dark conditions to break the physiological dormancy of the seeds. Following the initial germinations, the Petri dishes were stored in a growth chamber with a 24 ±2°C temperature and 16-hour photoperiod for 20 days.

***In vitro* callus induction.** For *in vitro* callus induction; hypocotyl fragments (cut to around 1 cm length) taken from 14-day old germinated plantlets were used as explants (Figs 1a, 1b, 1c). Hypocotyl explants were cultured in MS media with nine different PGR combinations, including BAP (2, 3, and 4 mg/L) and NAA (0.1, 0.5, and 1 mg/L). The calculated parameters (callus and shoot ratio, color and texture of callus, and shoot/explant) were recorded 35 days after the culture. Each glass jar contained 4–5 explants and 50 ml of medium.

***In vitro* callus proliferation.** For *in vitro* callus proliferation, calli obtained from hypocotyl explants were cut in 1 cm^3 pieces and cultured in MS media with four PGR combinations, including TDZ (0.5, 1, 2, and 3 mg/L) and NAA (0.2 mg/L) (experiment 1). Calli obtained from the first set of experiments (about 1.5 g

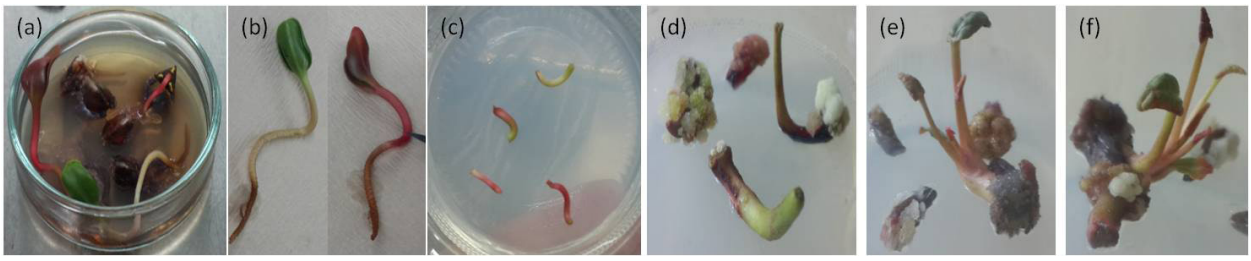

Fig. 1.a–c. *In vitro* germinated 14-days old *R. ribes* L. hypocotyls and their transfer to nutrient media; **d–f.** callus induction and shoot formations in Murashige and Skoog media supplemented with 4 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L naphthylacetic acid (NAA)

initial fresh weight) were cultured in 36 different PGR combinations, including BAP (1, 2, 3, and 4 mg/L) + IBA (0.2, 0.5, and 1 mg/L), BAP (1, 2, 3, and 4 mg/L) + NAA (0.2, 0.5, and 1 mg/L), and TDZ (1, 2, 3, and 4 mg/L) + NAA (0.2, 0.5, and 1 mg/L) (experiment 2). Each glass jar contained 5–6 explants and 50 mL of medium. After 35 days of culture, callus and shoot ratio (%), callus fresh weight, color and texture of callus, and shoot/explant were determined.

The pH of all the culture media used in the present experiments was adjusted to 5.8 using 1 N NaOH and 1 N HCl. The entire media was supplemented with 30 g/L sucrose and 7 g/L agar, and all were autoclaved at 121°C for 20 min. All cultures were maintained at 24 ±2°C, under a 16/8 h day/night photoperiod.

Data analysis. In all experiments a minimum of 3 repetitions were cultured. Each single treatment consisted of 4–6 explants per glass jar. The data was statistically analyzed using a one-way analysis of variance (ANOVA) test with Statgraphics statistical software, followed by Duncan's multiple range test comparisons for significant differences ($p < 0.01$). The values are represented means ±standard error (SE).

RESULTS

***In vitro* callus induction.** The callus induction ratios varied between 16.6–83.3% with the greatest value from the MS media including different concentrations of BAP (2, 3, and 4 mg/L) and 0.1 mg/L NAA. Shoot formations were also observed in the callus induction media, and this ratio varied between 0.0–16.7%. The greatest callus (83.3%) and shoot formation ratios (16.7%) were observed in the MS media, including 4 mg/L BAP and 0.1 mg/L NAA (Fig. 1d, 1e, 1f). Excepting for 3 MS media combinations (3 mg/L BAP + 0.1 mg/L NAA, 3 mg/L BAP + 1 mg/L NAA, and 4 mg/L BAP + 0.1 mg/L NAA), in all the other media combinations, a dominantly (%) whitish cream friable callus color was prominent (Tab. 1).

***In vitro* callus proliferation.** The results of the first set of experiments are provided in Table 2 (experiment 1). The differences in the investigated parameters of the PGR combinations were found to be significant ($p < 0.01$). The greatest callus (70.1%) and shoot formation ratio (6.3%) was obtained from the MS media supplemented with 1 mg/L TDZ and 0.2 mg/L NAA. Except for the 3 mg/L TDZ- and 0.2 mg/L NAA-containing PGR combinations, a dominantly whitish cream friable callus color was observed in all the PGR combinations (Tab. 2).

The results of the second set of experiments, in which 36 different PGR combinations were tested for callus proliferation, are provided in Table 3 (experiment 2). The differences in the investigated parameters of the PGR combinations were found to be signif-

Table 1. Effect of PGR on callus and adventitious shoot induction from hypocotyl explants of *R. ribes* L.

PGR (mg/L)	EC	Callus ratio (%)	Color and rate of callus (%)	Texture of callus	Shoot ratio (%)	Shoot/explant
BAP + NAA						
2 + 0.1	12	83.3 ±1.7 a	WC (60), LG (40)	F	0.0 ±0.0 c	0.0 ±0.0 c
2 + 0.5	12	33.3 ±2.0 c	WC (100)	F	0.0 ±0.0 c	0.0 ±0.0 c
2 + 1	12	50.0 ±1.2 b	WC (100)	F	0.0 ±0.0 c	0.0 ±0.0 c
3 + 0.1	12	83.3 ±1.6 a	DG (72.7), BR (27.3)	NF, F	8.3 ±0.8 b	0.6 ±0.2 a
3 + 0.5	12	33.3 ±0.9 c	WC (75), LG (25)	F	16.7 ±0.8 a	0.3 ±0.1 b
3 + 1	12	25.0 ±0.1 d	BR (100)	F	0.0 ±0.0 c	0.0 ±0.0 c
4 + 0.1	12	83.3 ±1.7 a	BR (40), LG (30), WC (30)	F	16.7 ±0.9 a	0.2 ±0.1 bc
4 + 0.5	12	16.6 ±0.7 e	WC (100)	F	8.3 ±0.8 b	0.1 ±0.1 bc
4 + 1	12	50.0 ±1.2 b	WC (100)	F	0.0 ±0.0 c	0.0 ±0.0 c
p value		0.000*			0.000*	0.000*

The means indicated with different small letters in the same columns are significantly different (Duncan's multiple range test, * $p < 0.01$). PGR – plant growth regulator, EC – the number of cultured explants, WC – whitish cream, LG – light green, DG – dark green, BR – brownish red, F – friable, NF – non-friable

Table 2. Effect of different PGR combinations on callus proliferation and adventitious shoot formation of the calli of *R. ribes* L. (1st experiment)

PGR (mg/L)	EC	Callus ratio (%)	Color and rate of callus (%)	Texture of callus	Shoot ratio (%)	Shoot/explant
TDZ + NAA						
0.5 + 0.2	50	57.2 ±1.2 c	WC (86.2), BR (13.8)	F	2.1 ±0.3 b	0.1 ±0.1 a
1 + 0.2	49	70.1 ±0.7 a	WC (71.4), BR (28.6)	F	6.3 ±0.5 a	0.2 ±0.1 a
2 + 0.2	44	62.5 ±1.9 b	WC (66), LG (30), BR (4)	F	0.0 ±0.0 c	0.0 ±0.0 b
3 + 0.2	47	55.4 ±0.7 c	DG (50), BR (50)	NF, F	2.1 ±0.2 b	0.1 ±0.1 a
p value		0.000*			0.000*	0.003*

The means indicated with different small letters in the same columns are significantly different (Duncan's multiple range test, * $p < 0.01$). PGR – plant growth regulator, EC – the number of cultured explants, WC – whitish cream, LG – light green, DG – dark green, BR – brownish red, F – friable, NF – non-friable.

Initial incubated size of callus was 1.0 cm^3

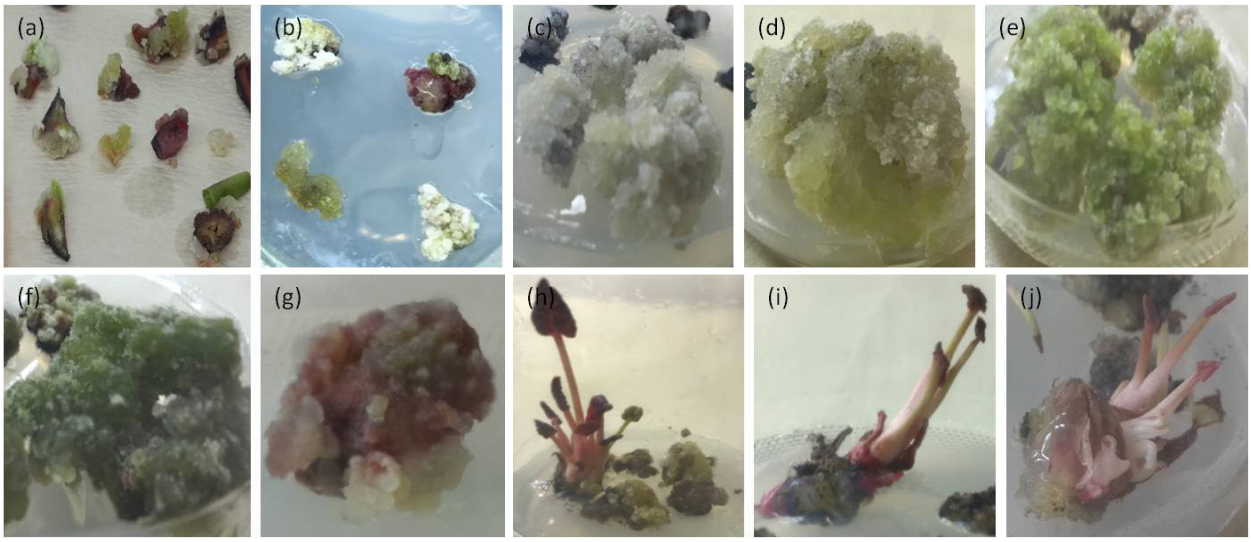

Fig. 2. Cultured calli: **a, b.** at the 35th day of culture; **c.** whitish cream (WC) colored callus; **d, e.** light greenish (LG) colored callus [4 mg/L thidiazuron (TDZ) + 0.2 mg/L naphthylacetic acid (NAA)]; **f.** dark greenish (DG) colored callus; **g.** brownish red (BR) colored callus; **h, i, j.** shoot formations [2 mg/L 6-benzylaminopurine (BAP) + 1 mg/L indole-3-butyric acid (IBA)]

icant ($p < 0.01$). The callus proliferation ratios varied between 12.5–100%. In terms of callus ratios (%), the MS media containing different concentrations of BAP and IBA were generally found to be more successful.

The most successful media (100%) were identified as 1 mg/L BAP + 0.5 mg/L IBA (2), 1 mg/L BAP + 1 mg/L IBA (3), 2 mg/L BAP + 0.2 mg/L IBA (4), 3 mg/L BAP + 0.2 mg/L IBA (7), 3 mg/L BAP + 1 mg/L IBA (9), and 4 mg/L BAP + 0.2 mg/L IBA (10) numbered combinations, including BAP and IBA as well as 1 mg/L TDZ + 0.2 mg/L NAA (25) and 2 mg/L TDZ + 0.2 mg/L NAA (28) numbered combinations including, TDZ and NAA (Tab. 3). The callus and shoot formations obtained from different PGR combinations are presented in Figure 2.

The callus fresh weights (FW) of the PGR combinations varied between 2.7–12.7 g, and TDZ and NAA–containing combinations were found to be more

Table 3. Effect of different combination PGR on callus proliferation and adventitious shoot formation from calli of *R. ribes* L. (2nd experiment)

Medium No	PGR (mg/L)	EC	Callus ratio (%)	Callus fresh weight (g)	Shoot ratio (%)	Shoot/explant
	BAP + IBA					
1	1 + 0.2	8	75.0 ±5.0 c	5.0 ±0.1 ij	0.0 ±0.0 d	0.0 ±0.0 d
2	1 + 0.5	10	100.0 ±1.1 a	6.8 ±0.4 g	0.0 ±0.0 d	0.0 ±0.0 d
3	1 + 1	10	100.0 ±1.2 a	6.7 ±0.5 gh	0.0 ±0.0 d	0.0 ±0.0 d
4	2 + 0.2	10	100.0 ±0.8 a	4.3 ±0.2 jk	0.0 ±0.0 d	0.0 ±0.0 d
5	2 + 0.5	9	57.5 ±2.5 de	6.8 ±0.2 g	10.0 ±0.4 c	1.2 ±0.2 b
6	2 + 1	10	40.0 ±5.0 hi	4.8 ±0.3 ij	22.5±2.5 a	1.6 ±0.1 a
7	3 + 0.2	10	100.0 ±0.8 a	4.9 ±0.4 ij	0.0 ±0.0 d	0.0 ±0.0 d
8	3 + 0.5	8	75.0 ±1.1 c	4.8 ±0.7 ij	0.0 ±0.0 d	0.0 ±0.0 d
9	3 + 1	9	100.0 ±1.4 a	5.2 ±0.2 ij	0.0 ±0.0 d	0.0 ±0.0 d
10	4 + 0.2	9	100.0 ±0.4 a	2.7 ±0.2 l	0.0 ±0.0 d	0.0 ±0.0 d
11	4 + 0.5	9	45.0 ±5.0 gh	5.1 ±0.1 ij	22.5±2.5 a	0.4 ±0.2 c
12	4 + 1	10	40.0 ±0.2 hi	2.9 ±0.1 l	20.0 ±2.2 b	0.5 ±0.2 c
	BAP + NAA					
13	1 + 0.2	8	25.0 ±5.0 l	2.8 ±0.2 l	0.0 ±0.0 d	0.0 ±0.0 d
14	1 + 0.5	8	37.5 ±2.5 ij	2.7 ±0.1 l	0.0 ±0.0 d	0.0 ±0.0 d
15	1 + 1	9	52.5 ±2.5 ef	5.7 ±0.2 hi	0.0 ±0.0 d	0.0 ±0.0 d
16	2 + 0.2	10	50.0 ±0.2 fg	4.7 ±0.3 ij	0.0 ±0.0 d	0.0 ±0.0 d
17	2 + 0.5	8	50.0 ±0.3 fg	4.3 ±0.1 jk	0.0 ±0.0 d	0.0 ±0.0 d
18	2 + 1	8	37.5 ±2.5 ij	4.7 ±0.2 ij	0.0 ±0.0 d	0.0 ±0.0 d
19	3 + 0.2	8	12.5 ±2.5 m	2.7 ±0.3 l	0.0 ±0.0 d	0.0 ±0.0 d
20	3 + 0.5	8	37.5 ±2.5 ij	2.8 ±0.1 l	0.0 ±0.0 d	0.0 ±0.0 d
21	3 + 1	8	50.0 ±0.3 fg	4.7 ±0.2 ij	0.0 ±0.0 d	0.0 ±0.0 d
22	4 + 0.2	9	35.0 ±5.0 i–k	3.5 ±0.1 kl	0.0 ±0.0 d	0.0 ±0.0 d
23	4 + 0.5	8	62.5 ±2.5 d	7.6 ±0.1 e–g	0.0 ±0.0 d	0.0 ±0.0 d
24	4 + 1	8	37.5 ±2.5 ij	6.8 ±0.1 g	0.0 ±0.0 d	0.0 ±0.0 d
	TDZ + NAA					
25	1 + 0.2	10	100.0 ±0.4 a	12.5 ±0.5 a	0.0 ±0.0 d	0.0 ±0.0 d
26	1 + 0.5	8	75.0 ±0.3 c	10.6 ±0.4 b	0.0 ±0.0 d	0.0 ±0.0 d
27	1 + 1	7	83.3 ±3.3 b	10.8 ±0.3 b	0.0 ±0.0 d	0.0 ±0.0 d
28	2 + 0.2	9	100.0 ±0.2 a	12.7 ±0.4 a	0.0 ±0.0 d	0.0 ±0.0 d
29	2 + 0.5	5	12.5 ±0.2 m	9.7 ±0.6 bc	0.0 ±0.0 d	0.0 ±0.0 d
30	2 + 1	6	16.7 ±1.4 m	7.2 ±0.1 fg	0.0 ±0.0 d	0.0 ±0.0 d
31	3 + 0.2	6	50.0 ±3.4 fg	9.5 ±0.1 cd	0.0 ±0.0 d	0.0 ±0.0 d
32	3 + 0.5	6	33.3 ±0.2 i–k	7.2 ±0.8 fg	0.0 ±0.0 d	0.0 ±0.0 d
33	3 + 1	8	50.0 ±1.7 fg	8.6 ±0.5 de	0.0 ±0.0 d	0.0 ±0.0 d
34	4 + 0.2	5	29.1 ±4.2 kl	10.2 ±0.1 bc	0.0 ±0.0 d	0.0 ±0.0 d
35	4 + 0.5	7	32.5 ±2.5 jk	8.0 ±0.1 ef	20.0 ±2.9 b	0.1 ±0.1 d
36	4 + 1	6	12.5 ±0.5 m	5.3 ±0.1 ij	0.0 ±0.0 d	0.0 ±0.0 d
	p value		0.000*	0.000*	0.000*	0.000*

The means indicated with different small letters in the same columns are significantly different (Duncan's multiple range test, * $p < 0.01$). PGR – plant growth regulator, EC – the number of cultured explants, BAP – 6-benzylaminopurine, IBA – indole-3-butyric acid, NAA – naphthylacetic acid, TDZ – thidiazuron

Initial incubated fresh weight (FW) of callus was 1.5 g

successful (Tab. 3). The greatest callus fresh weights were obtained on the media containing 2 mg/L TDZ + 0.2 mg/L NAA (12.7 ±0.4 g) and 1 mg/L TDZ + 0.2 mg/L NAA (12.5 ±0.5 g) PGR combinations. Shoot formation ratios (%) varied between 0–22.5% and number of shoots per explant varied between 0–1.6 shoots. For shoot formation, the media containing BAP- and IBA-media combinations were found to be more successful (Tab. 3).

DISCUSSION

Previous researchers have used different explant types and MS media combinations for *in vitro* callus and shoot regeneration in *Rheum* species. Wang et al. [2011] reported that the highest callus induction (58.3%) from the leaf explants of *Rheum franzenbachii* Munt. was obtained from MS medium supplemented with 2 mg/L BAP and 0.5 mg/L NAA. Other reports also indicated that the combination of 0.5 mg/L TDZ and 0.2 mg/L NAA was the most suitable for callus fresh weight (13.15 g). In *Rheum ribes* L., the most successful results in terms of callus regeneration rate were obtained from *in vitro* developed hypocotyl explants with MS + 1 mg/L BAP + 1 mg/L IBA (88.8%) and MS + 2 mg/L BAP + 1 mg/L IBA media (83.3%) [Tuncer and Günsan 2017]. In another study conducted on *Rheum ribes* L., hypocotyl explants taken from 5-days old plantlets developed under in *vitro* conditions were used and callus regeneration was achieved in 1 mg/L BA- and 1 mg/L IBA-containing MS media [Farzami Sepehr and Ghorbanli 2002].

Similar studies show that the greatest callus regeneration from rhizome explants of *R. coreanum* Nakai was obtained from MS media supplemented with 2,4-D (0.2–0.3 mg/L) [Mun and Mun 2016]. Another study conducted on *Rheum webbianum* Royle shows that the greatest callus regeneration (100%) from rhizome explants was achieved on MS media supplemented with 0.5 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) supplemented MS media during a 30--days culture [Rashid et al. 2014]. In *Rheum spiciforme* Royle, the greatest callus regeneration (80%) was obtained with MS + 15 µM BAP + 10 µM 2,4-D + 10 µM IBA media, and the callus fresh weights of this media varied between 5–7 g [Tabin et al. 2016].

In the second set of experiments for the callus proliferation of *Rheum ribes* L., the combinations of the different ratios of BAP and IBA were found to be more successful, and some media combinations (2, 3, 4, 7 9, and 10 numbered combinations) yielded 100% callus regeneration (Tab. 3). The present findings of callus regeneration ratios (%) were greater than the values obtained for the *R. ribes* L. obtained by Tuncer and Günsan 2017 and other species of *Rheum* such as *R. franzenbachii* Munt. and *R. spiciforme* Royle [Wang et al. 2011, Tabin et al. 2016]. In terms of callus proliferation and fresh weight, the media supplemented with TDZ- and NAA PGR combinations were found to be more successful than the other PGR combinations. The callus fresh weights obtained on the media with TDZ and NAA combinations varied between 5.3 ±0.1 and 12.7 ±0.4 g, and significant increases were observed when compared to the initial callus fresh weight – 1.5 g (Tab. 3). The findings of the current study are in agreement with the findings reported by Wang et al. [2011].

In previous *in vitro* shoot regeneration studies on *Rheum* species, shoot regeneration ratios varied depending on culture media composition, explant type and species. Tuncer and Günsan [2017] worked on hypocotyl explants of *Rheum ribes* L. and reported the greatest number of shoots per explants (4 ±0.89 shoot) for 2 mg/L BAP and 1 mg/L IBA supplemented MS media. In another study conducted on *Rheum coreanum* Nakai, the greatest shoot regeneration ratio from the calli developed under *in vitr*o conditions was obtained on the MS media containing BAP (2 mg/L) and NAA (0.2 mg/L) [Mun and Mun 2016]. Studies have been carried out also on the endangered medicinal plant *Rheum emodi* Wall., and it was revealed that the most efficient MS media combination was 15 µM BAP + 15 µM IBA [Tabin et al. 2018]. The greatest shoot regeneration from rhizome explants of *Rheum emodi* Wall. was obtained from liquid and solid MS media supplemented with 10 µM BAP and 5 µM IBA [Malik et al. 2010].

Kozak and Sałata [2011] worked with shoot bundles of *Rheum rhaponticum* L. grown under aseptic conditions and reported the greatest shoot regeneration ratio for MS media supplemented with BA (11.1–22.2 µmol/dm^3). In an endangered medicinal plant,

Rheum webbianum Royle, the maximum shoot regeneration (2.8 ±0.2 shoot) from rhizome explants was obtained from MS + BAP (5 mg/L) + IAA (2 mg/L) media on the 16th day of the culture [Rashid et al. 2014]. In *Rheum spiciforme* Royle, the greatest shoot regeneration from leaf explants was obtained on MS media fortified with 15 µM BAP and 15 µM 2,4-D [Tabin et al. 2016].

In the present study, when BAP- and IBA-combinations were added to MS media, shoot regeneration from calli was more successful than other PGR combinations for *R. ribes* L., and the greatest shoot regeneration (22.5%) and shoot per explant (1.6 shoot) were achieved with the MS + BAP (2 mg/L) + IBA (1 mg/L) media (Tab. 3). The present findings are in agreement with the results reported by Tuncer and Günsan [2017], who conducted a study on the same species.

CONCLUSION

In this study, an efficient callus proliferation protocol was developed on *R. ribes* L. under *in vitro* conditions. As a result, it was determined that different combinations BAP and IBA added to MS medium were the most suitable for callus induction and shoot regeneration while combinations containing low-dose TDZ (especially 1 mg/L) and NAA (0.2, 0.5, 1 mg/L) were more successful in terms of callus fresh weight. However, further research is recommended to be carried out to develop efficient protocols for *in vitro* shoot and root regeneration of *R. ribes* L.

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