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Optimizing culture media for in vitro microbulb production of medicinal wild plant: *Eremurus spectabilis* M. Bieb.

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ABSTRACT

Eremurus spectabilis M. Bieb. is an important wild plant species consumed as a vegetable. The plant is also used for medicinal purposes, in the cut flower industry as ornamental geophytes. In this study, it was aimed to ensure germination, bulblet regeneration, bulblet size enlargement in *E. spectabilis* under in vitro conditions. Gamborg (B5) and White (WH) media were used for in vitro germination of seed tip-cutting and total of 24 Murashige and Skoog (MS) combinations supplemented with thidiazuron (TDZ) (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + naphthalene acetic acid (NAA) (0, 0.1, and 0.5 mg L^{-1}) and 6-benzyl amino purine (BAP) (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + NAA (0, 0.1, and 0.5 mg L^{-1}) were used for bulblet regeneration experiments. For bulblet size enlargement, two trials were set up with an interval of 30-35 d. The highest germination ratio (61.5%) was obtained from B5 medium ($p < 0.01$). In in vitro bulblet regeneration trials, MS media supplemented with 0.5 mg L^{-1} TDZ with 0.1 (65.8%) and 0.5 mg L^{-1} NAA (65.3%) and the combination of 1.0 mg L^{-1} TDZ with 0.5 mg L^{-1} NAA (65.1%) yielded more successful in terms of bulblet ratio ($p < 0.01$). For bulblet size enlargement, as the average of both trial sets, 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA combination was more successful in terms of bulblet ratio (79.05%) and bulblet diameter (2.05 cm). The most successful combinations in terms of average number of bulblets were 2.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (32.25 bulblets) and 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (30.6 bulblets), respectively.

Key words: 6-Benzyl amino purine, bulblet, edible wild plant, *Eremurus*, in vitro propagation, naphthalene acetic acid, thidiazuron.

INTRODUCTION

Eremurus spectabilis M. Bieb. is a perennial, herbaceous and bulbous wild edible species belonging to Liliaceae family. Due to high nutritional value of the plant, shoots and leaves are consumed as vegetable. The plant, which is also of medical importance (Pourfarzad et al., 2014; Abubaker and Hidayat, 2015; Tuzcu et al., 2017), is used as an ornamental geophyte in cut flower industry (Schiappacasse et al., 2013; Ahmad et al., 2014), as well as in the industry to obtain natural gum by drying and grounding the roots into powder form (Eghtedarnejad and Mansouri, 2016).

In vitro plant tissue culture techniques are widely used for conservation and reproduction of rare endemic species under the danger of extinction in habitat, medicinal, aromatic, and economically important species. In in vitro regeneration studies, once the appropriate protocol was developed for related species, it is possible to perform rapid in vitro regeneration of that species. In vitro regeneration studies have also been conducted in different species of the Liliaceae family, including the *Allium* spp. (Alizadeh et al., 2013; Kizil et al., 2014; Chauhan et al., 2015; Kwon et al., 2018), *Lilium* spp. (Dhyani et al., 2014), and *Fritillaria* spp. (Cakmak et al., 2016). In these studies, different in vitro bulblet formation ratios were achieved based on the species and the nutrient media combinations.

Present literature review revealed that there were only three studies in the world on in vitro regeneration of *Eremurus* species. The first study by Tuncer (2017) was conducted on three different wild species (*Ferula orientalis* L., *Chaerophyllum macrospermum* (Willd. ex Spreng.) Fisch. & C.A. Mey. ex Hohen, and *E. spectabilis*). The researcher cultured leaf and rhizome explants of *E. spectabilis* in MS (Murashige and Skoog, 1962) medium containing only combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5-2.0 mg L^{-1}) or 6-benzyl amino purine (BAP) (1.0-2.0 mg L^{-1}) + 2,4-D (0.5-2.0 mg L^{-1}) and reported no shoots from any medium combinations.

In the second study, the researcher cultured hypocotyl explants (0.5-1.0 cm) of in vitro-germinated 35-40 d old plantlets in MS media containing 36 different hormone combinations of 2,4-D (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + kinetin (0.5 mg L^{-1}), thidiazuron (TDZ) (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + naphthalene acetic acid (NAA) (0, 0.1, 0.5, and 1.0 mg L^{-1}), BAP (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + 2,4-D (0, 0.1, 0.5, and 1.0 mg L^{-1}) to stimulate bulb and/or shoot formation of *E. spectabilis* under in vitro conditions. The best results in terms of germination rate (57.49%) of tip-cut seeds were obtained from Gamborg (B5) medium. It was stated that 100% bulblet formation was observed in MS media with TDZ (0.5 mg L^{-1}) and NAA (0.5 and 0.1 mg L^{-1}) combinations, followed by only 0.5 mg L^{-1} BAP-containing (81.3%) media combinations. The researcher also stated that it would be beneficial to try different hormone combinations to increase bulblet size in in vitro regeneration studies of this species (Tuncer, 2020).

In another recent study, tuberous root explants of *E. spectabilis* were cultured in MS medium. The highest callus formation per explant (76.67%) was obtained from Murashige and Skoog (MS) medium supplemented with 10.0 mg L^{-1} 6-benzylaminopurine (BAP), and the highest shoot regeneration was obtained from MS or Schenk and Hildebrandt (SH) medium containing 2 mg L^{-1} BAP. Researchers also stated that the best rooting medium composition is 1/2 MS + 4.0% sucrose + 2.0 mg L^{-1} indole butyric acid (IBA) + 200 mg L^{-1} activated charcoal (Basiri et al., 2022).

The present study is somehow a follow up study of Tuncer (2020). In this study, besides prominent MS nutrient media combinations of Tuncer (2020), different MS media combinations were also included in experimental design. In this way, rapid in vitro bulblet regeneration potentials were investigated and bulblet size enlargement was tried to be achieved under in vitro conditions. Within the scope of this study, in vitro germination of *E. spectabilis* seeds, in vitro bulblet regeneration, and bulblet size enlargement were investigated.

MATERIALS AND METHODS

Plant materials and seed sterilization

The seeds of *Eremurus spectabilis* M. Bieb. were collected from natural habitat Gürpınar town (38°8'20.75'' N, 43°30'55.15'' E; 1730 m a.s.l.) of Van province, Turkey. To remove fungal disease agents, seeds were kept in 0.3% benomyl solution for 1 h, then washed through distilled water and kept in distilled water for 1 h. Then, to prevent possible bacterial infections, seeds were taken into a sterile planting cabinet, kept in 70% ethyl alcohol for 5 s and washed three times with bi-distilled water. Finally, seeds were kept in 40% sodium hypochlorite solution containing a few drops of Tween 20 for 10 min, then rinsed through bi-distilled water three times for 5 min and sterilization process was completed (Tuncer, 2020).

In vitro germination and explant preparation

In previous studies of Tuncer (2020) and Akdag and Tuncer (2020a), germination trials with tip-cut seeds were reported as the most effective treatments for elimination of dormancy encountered in *E. spectabilis* seeds. Therefore, in this study, tips of sterilized seeds were cut off, then the seeds were germinated in B5 (Gamborg et al., 1968) and WH (White, 1943) nutrient media under in vitro conditions. Germination media were supplemented with 7 g L^{-1} agar, 20 g L^{-1} sucrose, 0.75 g L^{-1} gibberellic acid (GA_3) and 50 mg L^{-1} citric acid and media pH was adjusted to 5.8. Petri dishes were wrapped with parafilm and kept in a refrigerator (4 °C) until the first germinations (approximately 20-25 d after seeding) to break the physiological dormancy of the seeds. Then, petri dishes were taken into an incubator set at 13-14 °C and kept there until the end of the germination period. At the end of the germination trials, germination ratio (%), mean germination time (d) and germination speed (d) were calculated. Hypocotyl fragments (cut in 0.5-1.0 cm long) taken from sterile plantlets germinated under in vitro conditions were used as explants. The following formulas were used in the calculations:

$$\text{Germination ratio (\%)} = (G/T) \times 100$$
$$\text{Mean germination time (d)} = \sum(n \times d)/\text{Total } G$$
$$\text{Germination speed (d)} = n1/t1 + n2/t2 + \ldots + nn/tn$$

where n is number of seeds germinated on each day, t is number of days for germination, G is germinated seed number; T is total number of seeds, d is number of days from the beginning of the test.

In vitro bulblet regeneration and bulblet size enlargement

For in vitro bulblet regeneration, explants were cultured in MS media containing 24 combinations of thidiazuron (TDZ) (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + naphthalene acetic acid (NAA) (0, 0.1, and 0.5 mg L^{-1}) and 6-benzyl amino purine (BAP) (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) + NAA (0, 0.1, and 0.5 mg L^{-1}) in a climate room at 25 °C and 16:8 h photoperiod. To increase bulblet size, two different trials were set up with 30-35 d intervals. In the first set of experiments, bulblets obtained under in vitro conditions were cut into pieces and cultured in 13 MS nutrient media combinations, which were found to be more successful among the above-mentioned 24 MS combinations. In the second set of experiments, nine MS nutrient media combinations, which were found to be prominent in the first set of experiments, were used. At the end of experiments, bulblet ratio (%), number of bulblets per explant, average number of bulblets and bulblet diameters (cm) were calculated.

Statistical analysis

The data were statistically analyzed using ANOVA with Statgraphics statistical software (Statgraphics Technologies, The Plains, Virginia, USA), followed by Duncan's multiple range test comparisons for significant differences. All experiments were also set up in a randomized blocks design.

RESULTS

In vitro germination trial

In vitro germination test results are provided in Table 1. The difference between nutrient media in terms of germination ratio (%) was found to be significant ($p < 0.01$). The greatest germination ratio (61.5%) was obtained from B5 media (Table 1).

Bulblet regeneration and bulblet size enlargement

In vitro bulblet regeneration results are presented in Table 2. In terms of all parameters examined, differences between media combinations were found to be significant ($p < 0.01$). The best bulblet formation ratio (%) was obtained from the MS medium containing 0.5 mg L^{-1} TDZ+0.1 mg L^{-1} (65.8%), followed by the media combination of 0.5 mg L^{-1} TDZ+0.5 NAA (65.3%) and 1.0 mg L^{-1} TDZ+0.5 mg L^{-1} NAA (65.1%). In terms of average number of bulblets, the most successful MS combination was identified as 1.0 mg L^{-1} TDZ+0.5 mg L^{-1} NAA (17.5 bulblets), followed by 0.5 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (12.3 bulblets) combination (Table 2). The highest number of bulblets per explant (2.1) was achieved in 1.0 mg L^{-1} TDZ+0.5 mg L^{-1} NAA MS media combination (Figures 1a-1d). The greatest bulblet diameter (0.59 cm) was obtained from the MS medium containing 4.0 mg L^{-1} TDZ, followed by the media combination of 0.5 mg L^{-1} TDZ+NAA (0.1 and 0.5 mg L^{-1}) and the MS medium containing only 2.0 mg L^{-1} BAP (0.54 cm) (Table 2).

Table 1. Effect of different media on in vitro germination of *Eremurus spectabilis*.

Media	Germination rate	Germination speed	Mean germination time
	%	d	d
Gamborg (B5)	61.5 ± 0.8a	2.3 ± 0.1	10.1 ± 0.9
White (WH)	51.3 ± 1.3b	1.9 ± 1.9	10.1 ± 0.4
P value	0.000**	ns	ns

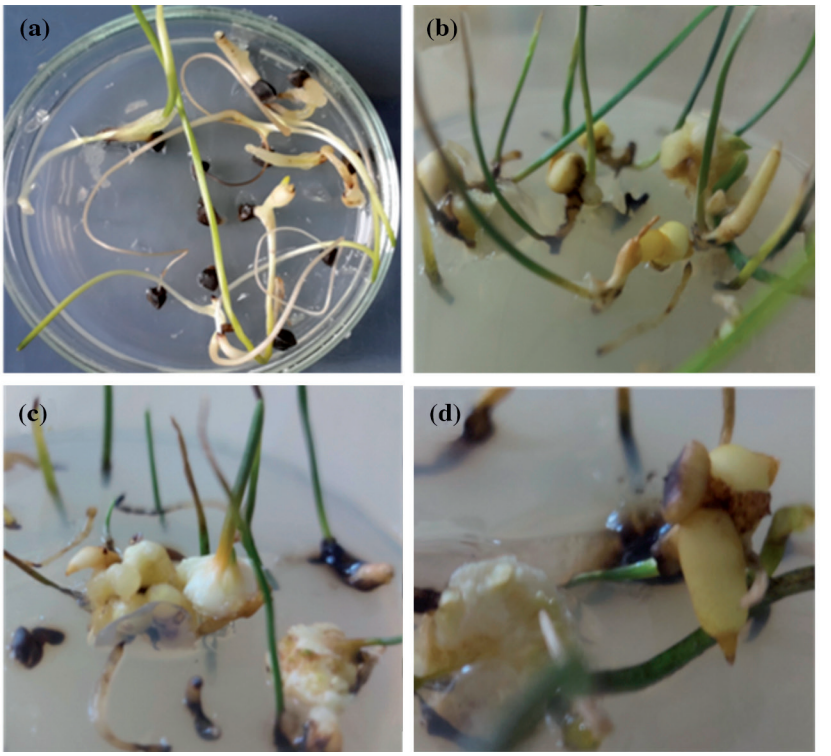
Means indicated with different small letters in the same column are significantly different (Duncan test, **$p < 0.01$, ns: nonsignificant).

Table 2. Effects of different MS media combinations on in vitro bulblet regeneration of *Eremurus spectabilis*.

Hormone treatment	CE	Bulblet ratio	Average nr of bulblets	Bulblets per explant	Bulblet diameter
mg L^{-1}	nr	%	nr	nr	cm
0.5 TDZ+0 NAA	34	33.6 ± 1.1h	5.6 ± 0.2g	1.6 ± 0.1c-e	0.30 ± 0.03f-h
0.5 TDZ+0.1 NAA	30	65.8 ± 1.7a	12.3 ± 0.9b	2.0 ± 0.1ab	0.54 ± 0.04ab
0.5 TDZ+0.5 NAA	30	65.3 ± 1.4a	7.6 ± 0.5f	1.3 ± 0.1e-h	0.54 ± 0.04ab
1.0 TDZ+0 NAA	30	23.1 ± 0.5jk	3.6 ± 0.2j-l	1.1 ± 0.1f-i	0.20 ± 0.02i
1.0 TDZ+0.1 NAA	45	52.1 ± 0.9e	9.0.±.0.4d-f	1.1 ± 0.1f-i	0.30 ± 0.03f-h
1.0 TDZ+0.5 NAA	56	65.1 ± 0.9a	17.5.±.1.1a	2.1 ± 0.1a	0.51 ± 0.01bc
2.0 TDZ+0 NAA	25	55.8 ± 0.6cd	10.1 ± 0.7cd	1.9 ± 0.2a-c	0.50 ± 0.01bc
2.0 TDZ+0.1 NAA	25	56.5 ± 0.6c	9.0 ± 0.5d-f	1.8 ± 0.2a-d	0.43 ± 0.03cd
2.0 TDZ+0.5 NAA	44	56.2 ± 1.2cd	8.5 ± 0.6ef	1.4 ± 0.2d-f	0.37 ± 0.01d-f
4.0 TDZ+0 NAA	30	53.1 ± 1.0de	10.5 ± 0.3c	1.3 ± 0.1e-h	0.59 ± 0.04a
4.0 TDZ+0.1 NAA	28	60.2 ± 1.1b	9.2 ± 0.5c-e	1.3 ± 0.2e-g	0.40 ± 0.03de
4.0 TDZ+0.5 NAA	31	56.3 ± 0.8cd	9.6 ± 0.6c-e	1.6 ± 0.1c-e	0.41 ± 0.03d
0.5 BAP+0 NAA	28	15.3 ± 0.3l	0.8 ± 0.3o	0.8 ± 0.1i	0.23 ± 0.02hi
0.5 BAP+0.1 NAA	33	25.0 ± 0.9ij	1.2 ± 0.2m-o	1.0 ± 0.1g-i	0.27 ± 0.02g-i
0.5 BAP+0.5 NAA	31	13.8 ± 0.4l	1.2 ± 0.3no	0.9 ± 0.1hi	0.30 ± 0.02f-h
1.0 BAP+0 NAA	28	35.8 ± 1.2h	2.5 ± 0.2l-n	1.1 ± 0.1f-i	0.23 ± 0.03hi
1.0 BAP+0.1 NAA	29	22.5 ± 1.0jk	1.5 ± 0.4m-o	0.7 ± 0.2i	0.28 ± 0.02gh
1.0 BAP+0.5 NAA	30	43.6 ± 1.4g	4.0 ± 0.4h-j	1.7 ± 0.2b-e	0.23 ± 0.01hi
2.0 BAP+0 NAA	25	61.1 ± 1.6b	3.8 ± 0.6j-l	2.0 ± 0.2ab	0.54 ± 0.02ab
2.0 BAP+0.1 NAA	30	27.1 ± 1.3i	2.8 ± 0.5k-m	1.0 ± 0.1g-i	0.27 ± 0.01g-i
2.0 BAP+0.5 NAA	28	14.1 ± 1.0l	1.2 ± 0.3m-o	0.8 ± 0.1i	0.32 ± 0.04e-g
4.0 BAP+0 NAA	27	48.3 ± 1.9f	4.8 ± 0.5g-i	1.6 ± 0.2c-e	0.44 ± 0.03cd
4.0 BAP+0.1 NAA	31	52.5 ± 1.5e	5.3 ± 0.4gh	1.3 ± 0.2e-g	0.43 ± 0.05cd
4.0 BAP+0.5 NAA	19	20.8 ± 0.7k	2.6 ± 0.5k-m	1.1 ± 0.1f-i	0.26 ± 0.02g-i
P value		0.000**	0.000**	0.000**	0.000**

Means indicated with different small letters in the same column are significantly different (Duncan test, **p < 0.01).
CE: Cultured explants, TDZ: thidiazuron; NAA: naphthalene acetic acid; BAP: 6-benzyl amino purine.

Figure 1. In vitro bulblets developed from hypocotyl explants in different MS media combinations: in vitro germinated in B5 media (a); 0.5 mg L^{-1} thidiazuron (TDZ) + 0.1 mg L^{-1} naphthalene acetic acid (NAA) (b); 1.0 mg L^{-1} TDZ + 0.5 mg L^{-1} NAA (c); and 0.5 mg L^{-1} TDZ + 0.5 mg L^{-1} NAA (d).



Two different set of experiments were conducted to increase bulblet size of *E. spectabilis* in 30-35 d interval. Results of the first set of experiments are given in Table 3. In the first set of experiments, differences between the nutrient media combinations were significant for all parameters ($p < 0.01$), except for average bulb diameter ($p < 0.05$). The highest bulblet regeneration (78.9%) was obtained from 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA media combination. In terms of average number of bulblet and number of bulblets per explant, the greatest values were reached again in media combinations of 2.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (37 bulblets, 4.9 bulblets per explant) and 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (32 bulblets, 3.4 bulblets per explant). The greatest bulblet diameter (2 cm) was obtained from the MS medium supplemented with 1.0 mg L^{-1} TDZ and 0.1 mg L^{-1} NAA (Table 3).

Results of the second set of experiments for bulblet size enlargement are summarized in Table 4. The bulblet regeneration ratios varied between 45.5%-79.2%. The greatest bulblet regeneration (79.2%) and average bulblet diameter (2.1 cm) were obtained from 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA combination. In terms of average number of bulblets, the greatest values (32.5 bulblets, 8.3 bulblets per explant) were obtained from the MS combination containing 2.0 mg L^{-1} TDZ+0.5 mg L^{-1} NAA (Table 4, Figures 2a-2e).

Table 3. Results for in vitro bulblet enlargement of *Eremurus spectabilis* (1st set of experiments).

Hormone treatment	CE	Bulblet ratio	Average nr of bulblets	Bulblets per explant	Bulblet diameter
mg L^{-1}	nr	%	nr	nr	cm
0.5 TDZ+0.1 NAA	33	55.9 ± 2.8d	9.3 ± 0.9ef	1.5 ± 0.3de	1.7 ± 0.1ab
0.5 TDZ+0.5 NAA	36	41.3 ± 1.1e	10.3 ± 0.8ef	2.8 ± 0.4bc	1.2 ± 0.1b-d
1.0 TDZ+0.1 NAA	32	78.9 ± 1.1a	32.0 ± 2.9b	3.4 ± 0.4b	2.0 ± 0.2a
1.0 TDZ+0.5 NAA	41	54.8 ± 1.2d	17.0 ± 1.1d	2.2 ± 0.4c-e	1.1 ± 0.3b-d
2.0 TDZ+0 NAA	44	23.8 ± 2.4f	5.3 ± 0.5g	2.3 ± 0.1c-e	1.4 ± 0.3b-d
2.0 TDZ+0.1 NAA	31	73.6 ± 0.9ab	37.0 ± 1.3a	4.9 ± 0.3a	1.5 ± 0.2a-d
2.0 TDZ+0.5 NAA	38	54.2 ± 1.6d	18.8 ± 1.4cd	2.7 ± 0.8bc	0.9 ± 0.1d
4.0 TDZ+0 NAA	39	55.2 ± 1.8d	7.8 ± 0.8fg	1.7 ± 0.3de	1.1 ± 0.1b-d
4.0 TDZ+0.1 NAA	41	42.9 ± 2.4e	11.5 ± 0.6e	1.3 ± 0.1e	1.0 ± 0.2cd
4.0 TDZ+0.5 NAA	40	72.5 ± 3.2b	16.5 ± 0.6d	2.3 ± 0.1cd	1.5 ± 0.2a-c
2.0 BAP +0 NAA	35	54.3 ± 0.5d	15.5 ± 1.0d	3.0 ± 0.3bc	1.4 ± 0.2b-d
4.0 BAP +0 NAA	35	64.0 ± 1.8c	11.0 ± 1.1ef	1.7 ± 0.2de	1.1 ± 0.1b-d
4.0 BAP +0.1 NAA	40	75.8 ± 1.5ab	22.3 ± 1.3c	2.8 ± 0.1bc	1.0 ± 0.2cd
P value		0.000**	0.000**	0.000**	0.0175*

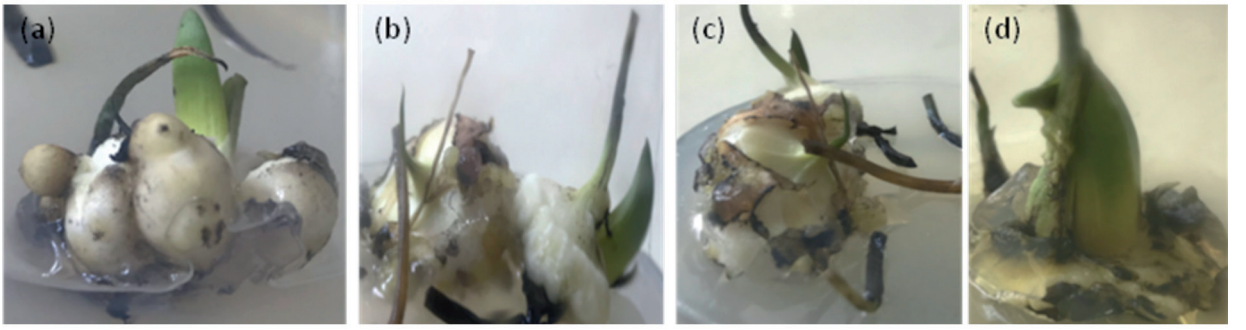
Means indicated with different small letters in the same column are significantly different (Duncan test, **p < 0.01, *p < 0.05).
CE: Cultured explants, TDZ: thidiazuron; NAA: naphthalene acetic acid; BAP: 6-benzyl amino purine.

Table 4. Results of the second set of experiments for bulblet size enlargement of *Eremurus spectabilis*.

Hormone treatment	CE	Bulblet ratio	Average nr of bulblets	Bulblets per explant	Bulblet diameter
mg L^{-1}	nr	%	nr	nr	cm
1.0 TDZ+0.1 NAA	24	79.2 ± 2.9a	29.2 ± 1.1ab	5.8 ± 0.9bc	2.1 ± 0.2a
1.0 TDZ+0.5 NAA	23	51.6 ± 1.5d	22.0 ± 1.6d	6.1 ± 0.6bc	1.4 ± 0.2bc
2.0 TDZ+0.1 NAA	29	66.6 ± 1.6b	27.5 ± 1.0bc	5.3 ± 0.5bc	1.5 ± 0.2bc
2.0 TDZ+0.5 NAA	26	58.9 ± 2.3c	32.5 ± 2.1a	8.3 ± 0.9a	1.1 ± 0.1c
4.0 TDZ+0.1 NAA	28	49.3 ± 1.2de	16.8 ± 1.6e	4.6 ± 0.8cd	1.1 ± 0.2c
4.0 TDZ+0.5 NAA	29	67.8 ± 1.9b	29.0 ± 1.3ab	5.1 ± 0.9bc	1.7 ± 0.1ab
2.0 BAP+0 NAA	23	45.5 ± 1.5e	13.8 ± 1.6ef	4.3 ± 0.5cd	1.5 ± 0.1bc
4.0 BAP+0 NAA	18	54.1 ± 0.9cd	11.0 ± 0.9f	2.7 ± 0.5d	1.2 ± 0.1bc
4.0 BAP+0.1 NAA	18	66.6 ± 1.2b	23.7 ± 1.3cd	6.8 ± 0.3ab	1.1 ± 0.1c
P value		0.000**	0.000**	0.0006**	0.0083**

Means indicated with different small letters in the same column are significantly different (Duncan test, **p < 0.01).
CE: Cultured explants, TDZ: thidiazuron; NAA: naphthalene acetic acid; BAP: 6-benzyl amino purine.

Figure 2. In vitro bulblet size enlargement of *Eremurus spectabilis*: 1.0 mg L^{-1} thidiazuron (TDZ) + 0.1 mg L^{-1} naphthalene acetic acid NAA (a-b), 2.0 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA (c), 1.0 mg L^{-1} TDZ + 0.5 mg L^{-1} NAA (d).



DISCUSSION

Sterile plantlets can be obtained under laboratory conditions by in vitro germination of seeds. It is important to establish healthy experiments with low risk of infection with explants taken from sterile plantlets, to ensure in vitro regeneration of that species. In a study, *E. spectabilis* seeds were germinated in three different nutrient media (MS, WH, and B5) with or without 1500 ppm GA_3 supplementation and reported very low germination rate (0.83%-1.50%) only in WH medium (Keskiner, 2017). In another study, *E. spectabilis* seeds were germinated in three different nutrient media (MS, WH, and B5) and kept the seeded petri dishes at 4 °C for different periods (30, 50, 80, and 100 d) until the beginning of germination. The greatest germination ratio (5.88%) was obtained from 100 d moist-cold stratification MS media (Akdag, 2019). Besides, both Keskiner (2017) and Akdag (2019) pointed out that very low germination was achieved under in vitro conditions.

Severe physiological dormancy is encountered in the seeds of *Eremurus* species and seeds require a long period of chilling to germinate (Keskiner, 2017; Akdag, 2019; Keskiner and Tuncer, 2019; Akdag and Tuncer, 2020b). Following these studies, another study was planned by Akdag and Tuncer (2020a) to shorten long chilling period for seeds to germinate and to increase germination and emergence performance. In that study, *E. spectabilis* seeds were subjected to moist-cold stratification treatments combined with different treatments (5 mM potassium nitrate for 24 and 48 h, 5 mM calcium chloride for 24 and 48 h, and seed tip-cutting). Researchers indicated that high germination ratios were obtained from tip-cut seeds subjected to cold moist stratification treatments for less than 30 d (20-25 d). As a result of this study by Akdag and Tuncer (2020a), the severe physiological dormancy encountered in *E. spectabilis* seeds was significantly reduced by physical treatment of cutting the seed tips and an opportunity was created to conduct in vitro germination studies with tip-cut seeds. In another study, B5 and WH media were used for in vitro germination of seeds and the greatest germination ratio (61.5%) was obtained from B5 medium (Tuncer, 2020). Present findings obtained from in vitro germination experiments were found to be compatible with the results of Tuncer (2020) and Akdag and Tuncer (2020a).

Although several in vitro regeneration studies have been carried out in many species belonging to Liliaceae family including *E. spectabilis*, the results obtained from these studies were not concordant because of species diversity (Alizadeh et al., 2013; Dhyani et al., 2014; Kizil et al., 2014; Chauhan et al., 2015; Cakmak et al., 2016; Kwon et al., 2018). Therefore, present findings were discussed with the results of limited number of studies on in vitro regeneration of *Eremurus* species. There are only three studies in the world on in vitro regeneration of *Eremurus* species (Tuncer, 2017; Tuncer, 2020, Basiri et al., 2022). In the first study, the researcher cultured leaf and rhizome explants of *E. spectabilis* in different MS media compositions (0.5-2.0 mg L^{-1} 2,4-D; 1-2 mg L^{-1} BAP+0.5-2.0 mg L^{-1} 2,4-D), but reported that shoots could not be obtained from any media compositions and explant types (Tuncer, 2017). Such negative outcomes were mainly attributed to a quite large number of wild plant species included in the study, limited number of MS media combinations and intense infection especially in cultures where rhizomes collected from the field were used as explants.

To eliminate the negative issue encountered in the study of Tuncer (2017), another comprehensive study was conducted by the same researcher (Tuncer, 2020). In that study, the researcher set up in vitro germination experiments with tip-cut seeds to stimulate bulblet formation of *E. spectabilis* and cultured hypocotyl explants taken from 35-40 d old germinated

sterile plantlets in 36 different hormone combinations of MS media. The highest germination ratio (57.5%) was obtained from B5 medium. It was reported that bulblet formation rates varied with the MS media combinations and the greatest rate (100%) was obtained from the combinations containing TDZ (0.5 mg L^{-1}) and NAA (0.5 and 0.1 mg L^{-1}) and the media containing only 0.5 mg L^{-1} BAP (81.3%). In the study conducted by Tuncer (2020), besides testing several media combinations, initiation of experiments with the explants taken from sterile plantlets developed under in vitro conditions affected the success positively since it both prevented infections and increased the rate of bulblet formation.

As suggested by Tuncer (2020), combinations containing BAP and NAA as well as TDZ and NAA combinations were included in present experiments. In addition, unlike the study of Tuncer (2020), in present study, various bulblet regeneration parameters were evaluated comprehensively. In our study, the greatest bulblet formation ratio (%) was obtained from the MS combination of 0.5 mg L^{-1} TDZ with 0.1 mg L^{-1} (65.8%) and 0.5 mg L^{-1} NAA (65.3%) and combination of 1.0 mg L^{-1} TDZ with 0.5 mg L^{-1} NAA (65.1%) (Table 2). Present successful combinations for bulblet formation rate (%) were similar with the combinations found to be successful by Tuncer (2020). In terms of average number of bulblets, the most successful combinations were identified as 1.0 mg L^{-1} TDZ+0.5 mg L^{-1} NAA (17.5 bulblets) and 0.5 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (12.3 bulblets), respectively (Table 2). The greatest average bulblet diameter was obtained from the MS medium containing 4.0 mg L^{-1} TDZ (0.59 cm), followed combination of 0.5 mg L^{-1} TDZ with NAA (0.1 and 0.5 mg L^{-1}) and only 2 mg L^{-1} BAP-containing MS medium (0.54 cm) (Table 2). When the examined bulblet growth parameters were evaluated in general, it was seen that combinations of low doses of TDZ (0.5 and 1.0 mg L^{-1}) with NAA (0.1 and 0.5 mg L^{-1}) yielded more successful outcomes. Besides, TDZ and NAA combinations were found to be more effective than BAP and NAA combinations in stimulation of bulblet formation from hypocotyl explants.

In the second part of the present study, as suggested by Tuncer (2020), experiments were conducted to increase the size of in vitro bulblet. As the average of two set of experiments, the most successful outcomes in terms of bulblet regeneration (average 79.05%) and bulblet diameter (average 2.05 cm) were achieved with 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA combination (Table 3-4). In terms of the average number of bulblets, 2.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (32.25 bulblets) and 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA (30.6 bulblets) were found to be more effective (Table 3-4).

CONCLUSIONS

In this report, an effective in vitro bulblet production protocol was developed in *Eremurus spectabilis*. The results of the study showed that thidiazuron (TDZ) + naphthalene acetic acid (NAA) combinations were found to be more effective than 6-benzyl amino purine (BAP) and NAA combinations for in vitro bulblet parameters. For in vitro bulblet regeneration, the most effective doses were the combination of low doses of TDZ (0.5 and 1.0 mg L^{-1}) with NAA (0.1 and 0.5 mg L^{-1}). For bulblet diameter, the most effective dose was 1.0 mg L^{-1} TDZ+0.1 mg L^{-1} NAA.

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