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Micropropagation of *Zinnia elegans* L.

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Abstract

In this study, *in vitro* and *in vivo* seed germination capacity of zinnia (*Zinnia elegans* L.), mostly grown as seasonal ornamental plant and the effects of different plant growth regulators on shoot regeneration and callus formation of different explants (whole leaf, leaf blade, leaf stalk) were investigated. The best surface sterilization of the seeds was achieved in 5% sodium hypochlorite solution for 3 min. The greatest germinations (25-30%) were observed in *in vivo* media. Among the *in vitro* germination media, MS medium yielded the worst outcomes and it was followed by White medium. Germination started five days after sowing, continued between the 5^{th} and 7^{th} days and stopped after the 7^{th} day. In terms of callus formation, tillering and shoot regeneration, MS medium yielded the best outcomes. Leaf explants were identified as the best source of explant. The best callus formation (2.67%) and shoot regeneration (2.33%) ratios were obtained from 1mg/L 2.4-D + 1mg/L BA containing MS medium. It was followed by 1mg/L 2,4-D (2,4 diklorofenoksi Asetik Asit) + 1mg/L BA (Benzil Adenin) containing B5 medium with shoot regeneration ratio of 2.25% and callus formation ratio of 1.13%.

Key words: *Zinnia elegans* L., *in vitro*, seed germination, micropropagation

Introduction

Ornamental plants are generally produced for esthetic, functional and economic purposes (Ay, 2009). Ornamental plant is a general term and include four sub-groups (cut flowers, indoor space (pot, hall) plant, outdoor space plants, natural flower bulbs (geophytes)) (Sayın and Sayın, 2004). However, emergence of ornamental plants as an economic sub-sector of plant production and integration of ornamental plants as a part of this sector in terms of production, marketing and employment coincide the end of 19^{th} and the beginning of 20^{th} century (Karagüzel et al., 2010).

Turkey exports ornamental plants to 52 countries of the world. Netherlands, United Kingdom, Eastern European countries and Balkan countries are the greatest export markets for cut flowers (Ay, 2009; Anonymous, 2010).

Zinnia elegans L. Naturally grows in Mexico. It is annual herbaceous plant. Flowering starts in July and continues until the initial frosts. Ornamental plant producers have to sow thousands of seeds and grow emergent plants to get 100 flowers with the same color and form (Rogers and Tjia, 1990). Therefore, tissue culture has become more advantageous for propagation from shoots, leaves and stalks.

This study was conducted to investigate the effects of different plant growth regulators and concentrations on adventive shoot regeneration of the explants from different sections of *in vitro* grown *Zinnia elegans* L. plants. In this way, *in vitro* protocol was developed for this species.

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Material and Method

Explants were taken from *Zinnia elegans* seedlings grown from the seeds. Bleach and 70% ethyl alcohol were used for sterilization of plant material. Emergence of radicula from the seed testa was considered as germination. Germinated seeds were counted daily throughout the culture at the same hour of the day. Emergence was considered as completed when the plantlets had parallel cotyledon leaves and emergence ratio was then calculated.

In both tissue culture and viol seedings, seed germination ratio (%), mean germination time (day) and mean emergence time (day) were investigated (Kayhan and Baran, 2014).

MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968) and WH (White, 1963) basic nutrient media were used in present experiments. The equipment to be used in tissue culture procedures were sterilized in an autoclave at 121 °C under 1 atm pressure for 1 h 30 min (Hatipoğlu, 1997). For seed gemination, MS and WH nutrient media were used. MS medium was supplemented with 30 g sucrose and WH medium was not supplemented with sugar.

Leaf blade and leaf stalk explants of germinated seeds were kept in 20% sodium hypochlorite solution for 10, 15, 20 and 30 min. Explants were planted into MS and B5 media containing different concentrations of BA (Benzil Adenin) and 2,4 D (2,4 Diklorofenoksi Asetik Asit). Cultures were incubated in a climate chamber at 25±2°C temperature, 1800 lux light intensity and 8-16 h photoperiod.

Statistical analysis

Descriptive statistics for the continuous variables were presented as Mean while count and percentages for categorical variables. One-way ANOVA was performed for the comparison of group means. Duncan multiple comparison test was also used to identify different groups. Statistical significance level was considered as 5% and SPSS (ver: 21) statistical program was used for all statistical computations.

Results and Discussion

Of 90 seeds sown into peat-filled viols, 85 geminated under controlled conditions of a climate chamber. Number of germinated plants and germination ratios in *in vitro* germination experiments in MS and White media are presented in Table 1.

Germination stages in petri dishes with MS and White media are presented in Figure 2 and 3.

Table 1. Number of germinated plants and germination ratio in MS and White media

	Media	19.12	20.12	21.12	22.12	23.12	24.12	25.12	26.12	27.12	28.12	Total	Percentage
Number of geminations	White	0	0	2.6	1.8	1	0.35	0	0	0	0	5.75	95.83
	MS	0	0	3.05	2.15	0.35	0	0	0	0	0	5.55	92.5
Germination ratio (%)	White			43.33	30	16.67	5.83	0	0	0	0		
	MS			50.83	35.83	5.83	0	0	0	0	0		

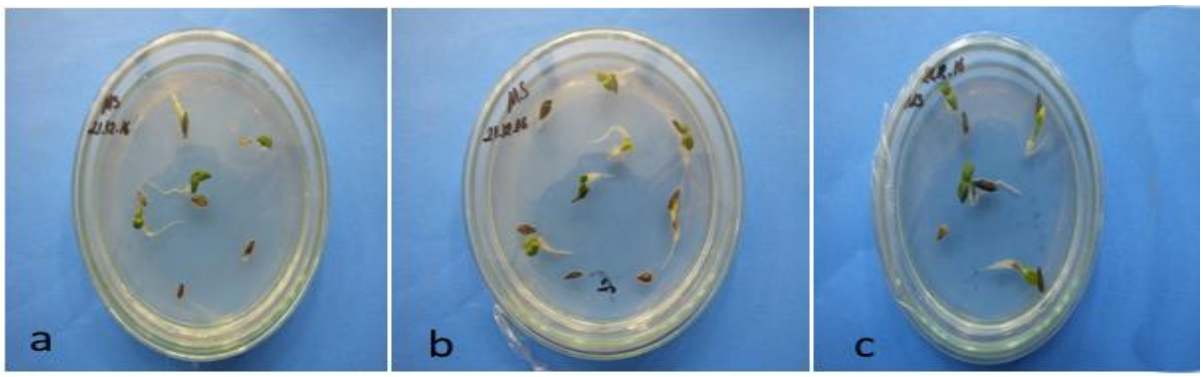

Figure 2. Germination in MS medium

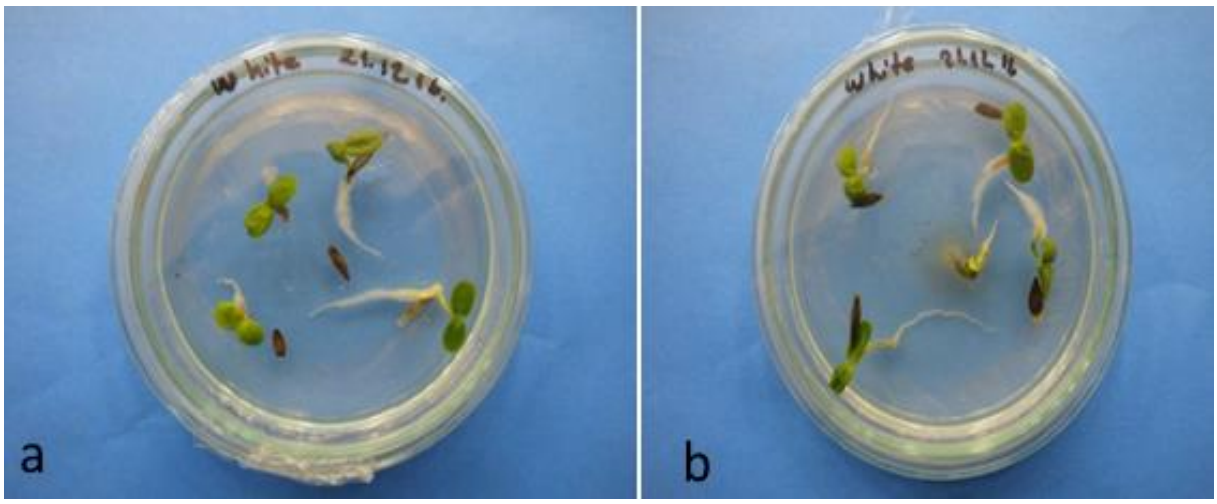

Figure 3. Germination in White medium

Descriptive statistics and comparison results for germination ratios of Zinnia flower seeds in 3 different media for 10 days are provided in Table 2.

Table 2. Descriptive statistics and comparison results

Date	in vivo		White		MS		General	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error		
19 A	0.000 A b	0.000	0.000 A c	0.000	0.000 A b	0.000	0.0000	0.000
20 A	0.000 A b	0.000	0.000 A c	0.000	0.000 A b	0.000	0.0000	0.000
21 A	0.000 B b	0.000	2.347 A a	0.124	3.073 A a	0.392	1.8067	1.435
22 A	0.000 B b	0.000	1.763 A ab	0.416	2.010 A a	0.488	1.2578	1.099
23 A	28.333 A a	0.667	0.980 B b	0.295	0.343 B ab	0.119	9.8856	13.853
24 A	1.000 A b	0.577	0.713 A b	0.645	0.000 A b	0.000	0.5711	0.872
25 A	0.667 A b	0.333	0.000 A c	0.000	0.000 A b	0.000	0.2222	0.441
26 A	0.000 A b	0.000	0.000 A c	0.000	0.000 A b	0.000	0.0000	0.000
27 A	0.000 A b	0.000	0.000 A c	0.000	0.000 A b	0.000	0.0000	0.000
28 A	0.000 A b	0.000	0.000 A c	0.000	0.000 A b	0.000	0.0000	0.000
Genel	3.000	1.571	0.580	0.167	0.543	0.198	1.3743	5.112

A, B, C→ : The dates indicated with different capital letters in the same column are significantly different ($p<0.05$)
a, b, c ↓ : The media indicated with different small letters in the same row are significantly different ($p<0.05$)

The differences in dates were assessed for each medium and the differences in media were assessed for each date. While the differences in germinations ratios were insignificant on 19, 20, 24, 25, 26, 27 and 28 December, the differences in the other dates were found to be significant. While there was no germination in *in vivo* medium on 21 and 22 December, germination ratios in the other two media varied between 1.76 - 3.07. The greatest germination (28.333) was observed in *in vivo* medium on 23 December and germination ratios in the other two media were lower.

Nutrient media had different effects on germination and germination ratios of the dates also varied with the media. Such a case indicated that media should be taken into consideration while finding out the proper germination dates and germination dates should be taken into consideration while selecting a nutrient medium.

Shoot and leaf explants of germinated plantlets in viols were taken into sub-cultures. In leaf explants, intense callus formation was observed especially in 1 mg/L 2,4-D+1 mg/L BA containing MS medium (Figure 4).

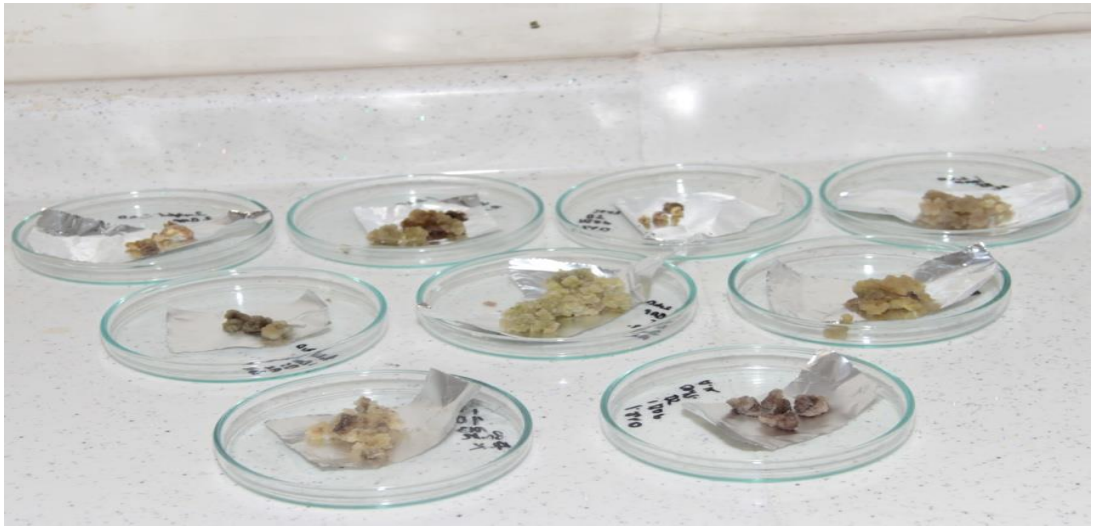

Figure 4. Callus formation in cotyledon explants

Leaf stalk and leaf blade explants of germinated seeds were cultured in 2 mg/L NAA+1 mg/L BA, 1 mg/L 2,4-D+1 mg/L BA, 2 mg/L 2,4-D+1 mg/L BA containing MS and B5 nutrient media. There were not any developments in 2 mg/L NAA+1 mg/L BA containing media. In 1 mg/L 2,4-D+1 mg/L BA containing MS medium, in six weeks, 2.67% shoot generation was achieved and 2.33% callus formation was observed. In 2 mg/L 2,4-D+1 mg/L BA containing MS medium, 1.75% shoot generation and 2.25% callus formation were achieved. In 1 mg/L 2,4-D+1 mg/L BA supplemented B5 medium, 2.25% shoot generation was achieved and 1.13% callus formation was observed. In term of actual shoot generation and callus formation, MS medium yielded better outcomes than B5 medium.

In 1 mg/L 2,4-D+1 mg/L BA containing medium, shoot lengths varied between 11 – 34 m, stem diameters varied between 0.66 - 1.82mm, total plant weights varied between 0.072 - 0.441g, number of leaf stalks varied between 1 – 8, number of cotyledons varied between 1 – 2, leaf widths varied between 1 - 5mm, leaf lengths varied 2 - 10mm, callus lengths varied between 5 – 22mm and callus weights varied between 0.03 - 0.34 mg (Table 3). Callus color was cream, white, grayish, greenish and brownish. Calluses were cream, off-white and white in color and hard until the 8^{th} week, then turned into greenish brown.

Table 3. Shoot characteristics

Height (mm)	Stem diameter (mm)	Total plant weight (g)	Number of leaves	Number of cotyledons	Leaf width (mm)	Leaf length (mm)	Callus length (mm)	Callus weight (mg)
20	1.09	0.297	8	2	2	5	15	0.23
12	1.14	0.126	6	2	1	2	7	0.07
29	1.71	0.201	8	2	1	3	7	0.13
31	1.07	0.432	8	2	2	3	22	0.23
24	1.12	0.441	1	1	5	10	10	0.30
11	0.98	0.123	6	2	3	4	14	0.20
15	0.66	0.195	6	2	2.5	10	6	0.06
34	0.96	0.075	8	2	2	4	5	0.04
22	1.01	0.072	6	2	2	4	11	0.03

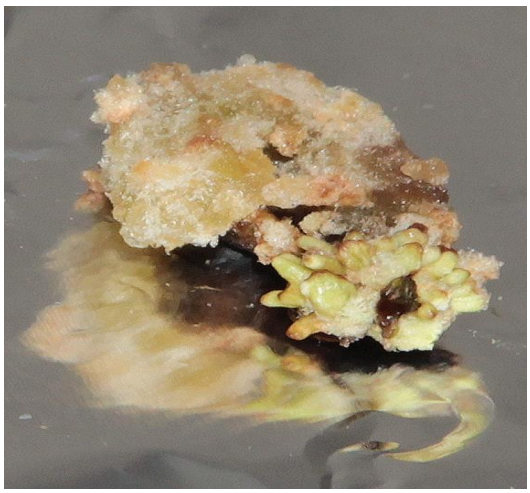

Figure 5. Shoot seen in calluses

The shoots obtained from 1 mg/L 2,4-D+1 mg/L BA had thinner light green leaves with a greater veiny appearance. Resultant adventive shoots tilled and generated one tiller in some shoots, more than two and up to 4 tillers in the others.

In terms of germination ratios, when the in vivo and in vitro germination success of seed explants in in vivo, WH and MS media were compared, it was observed that the best outcomes were achieved in in vivo medium and the worst outcomes were seen in MS medium. In terms of callus formation, MS medium yielded better outcomes than B5 medium. In terms of tillering and plant regeneration, again MS medium yielded better outcomes.

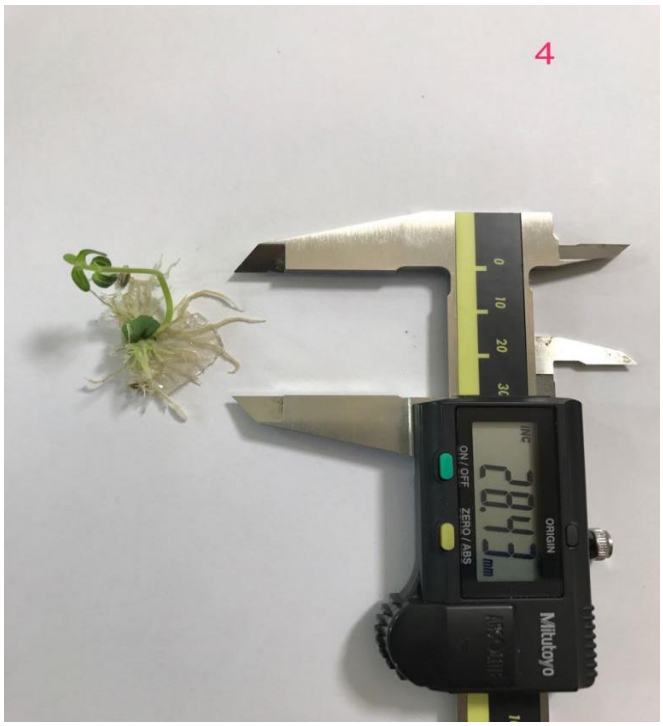

Figure 6. Length and rooting of shoots obtained from 1 mg/L 2,4-D+1 mg/L BA

Mahmodzadeh and Rohani (2010) conducted a study about in vitro germination of zinnia seeds, supplemented MS medium with 1-3 µM BAP and Kinetin and reported that kinetin supplementation to nutrient medium had positive effects on germination. The greatest germination ratio (90%) was observed in 1 µM Kinetin-containing MS nutrient medium. Present findings were not complying with the findings of that study since MS medium yielded the worst outcomes in present study. Such differences were mainly attributed to Kinetin supplementation into nutrient medium. Kinetin is a cytokinin group hormone and might have positive effects on germination (Özdemir et al., 2014).

In a previous study conducted with *Stevia rebaudiana* Bertoni of Asteraceae family, MS medium was identified as the most effective medium on germination. However, in present study, MS medium was identified as the worst medium in terms of germination. Although *Zinnia elegans* and *Stevia rebaudiana* Bertoni belong to the same family, such differences may be attributed to different genus of the species. Seed germination of requirements of germination of each species may be different from each other (Tandokazi, 2018).

In another study conducted with *Inula germanica* L., another species of Asteraceae family, seeds were germinated in 1 mg/l^{-1} BAP and 0.1 mg/ l^{-1} NAA containing MS media, but low germination ratios were observed. Such findings comply with the present findings. In present study, hormones were not supplemented into MS medium, but cytokinin and auxin hormone combinations were supplemented into MS medium in that study. In that case, hormone supplementations might have influenced endogenous hormone concentration of the plants, thus retarded germination. Hypocotyl sections of seedlings obtained from germinated *Inula germanica* L. seeds were cultured in MS medium to stimulate shoot regeneration and the greatest shoot regenerations (83.3%) was achieved with hypocotyl explants in MS medium. Those findings support the present findings, since the greatest shoot regeneration was also achieved in MS medium in present study (Trejgell, 2018).

Centaurea cineraria subsp. *circae* belongs to Asteraceae family. In a previous study conducted about *ex vitro* germination capacity of this species, the most efficient germination was achieved under *ex vitro* conditions rather than *in vitro* conditions. Dormancy was not observed in *Centaurea cineraria* subsp. *circae* seeds and 67.5% of sown seeds germinated (Valletta *et al*., 2016). These findings supported the present findings. However, although *Zinnia elegans* and *Centaurea cineraria* subsp. *circae* belong to the same family, they are different species. There were not any studies in literature about the micropropagation of *Zinnia elegans*. Therefore, present findings were mostly compared with the findings on other species of the same family.

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