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Response of Different Safflower Genotypes to Anther Culture

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ABSTRACT

In this study; the responses of 12 safflower genotypes to anther culture were determined in MS medium containing increasing doses of BAP (0, 0.5, 1, and 2 mg L^{-1}) and NAA (0, 0.1, and 0.5 mg L^{-1}). Donor plants were grown under field conditions and the main capitulas were collected in early or late uninucleate stage. A pre-treatment was applied to their capitules at +4 °C for 3-4 days. Only cvs. Balcı and Linas produced regenerable callus among these genotypes. Although regenerable callus rate was higher in Balcı than Linas, shoot regeneration was achieved only in Linas with 0.5 or 1 mg L^{-1} of BAP + 0.1 mg L^{-1} of NAA combinations. In total, 8 shoots of Linas were moved to ½ MS medium consisting of 0.1 mg L^{-1} of NAA, but rooting of them could not be performed. In sum, among the 12 safflower genotypes, cvs. Balcı and Linas have higher anther culture regeneration potential; however, it is necessary to focus on shoot development and rooting on these cultivars in further experiments.

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ÖZET

Bu çalışmada; 12 aspir genotipinin artan dozlarda BAP (0, 0.5, 1 ve 2 mg L^{-1}) ve NAA (0, 0.1 ve 0.5 mg L^{-1}) içeren MS ortamında anter kültürüne karşı tepkileri belirlenmiştir. Donör bitkiler tarla koşullarında yetiştirilmiş ve ana çiçek tablaları erken veya geç tek çekirdekli dönemde hasat edilmiştir. Ön işlem olarak +4 °C'de ve 3-4 gün süreyle soğuk muamelesi uygulanmıştır. Aspir genotiplerinden sadece Balcı ve Linas çeşitleri rejenere olabilir kallus üretmiştir. Balcı çeşidi daha yüksek oranda kallus oluşturmasına rağmen sadece Linas çeşidinde 0.5 veya 1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA kombinasyonlarından sürgün rejenerasyonu gerçekleşmiştir. Toplamda Linas çeşidine ait 8 sürgün 0.1 mg L^{-1} NAA içeren ½ MS ortamına aktarılmış, ancak köklenme sağlanamamıştır. Sonuç olarak; 12 aspir genotipi arasında Balcı ve Linas çeşitlerinin anter kültürü rejenerasyon potansiyelinin daha yüksek olduğu belirlenmiştir. Bununla birlikte, bu çeşitler üzerinde gelecekte sürgün geliştirme ve köklendirme çalışmalarına odaklanılması gerekmektedir.

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is used for different purposes from the stem to the seeds (Menegaes & Nunes, 2020). The rate of oil in newly registered safflower cultivars ranges from 35 to 50% (Rahamatalla et al., 2001) and approximately 90% of the oil consists of oleic and linoleic acids (Liu et al., 2016). Because safflower oil contains an average of 70% linoleic acid (omega 6) and is very rich in tocopherol (Matthaus et al., 2015), it is one of the alternative dietary vegetable oils that can be used for cardiovascular health. On the other hand, safflower cultivars containing of high oleic acid have been developed in recent years and thus, it has been contributed to the production of environmentally friendly biodiesel, just like canola.

It is necessary to develop new varieties that are more resistant to drought and cold, have high seed and oil yield, and have high linoleic and oleic acid content in oil to cultivate safflower in larger areas. Safflower is a

highly self-pollinated, but low level of cross-pollination occurs depending on variety, which indicates anther dehiscence after style elongation, and insect density (Pandey & Kumari, 2008).

Due to the pollination biology of safflower, very long periods are needed for pure-line selection after hybridizations. The application of haploidization techniques such as anther culture, which enables the selection of pure lines in a short time provides the shortening of the breeding period. However, due to regeneration and rooting problems, there have been two researches with Indian originated genotypes on anther culture in safflower so far (Prasad et al., 1990; Prasad et al., 1991). In these studies, it has been reported that the haploid regeneration rate varied according to the genotype, growth conditions of the donor plant, type of nutrient media, cold pre-treatment and the combinations of auxin and cytokinin in the medium.

Optimization of haploid regeneration protocols of safflower inbred lines that tolerate different stress conditions is very important for the production of new safflower cultivar in a short time in the future. Although this method is a precise, efficient and innovative method for safflower breeding, but haploid plant production differs according to genotypes. In this study, the responses of twelve safflower genotypes, especially selected for winter hardiness, to anther culture were investigated.

MATERIALS and METHODS

In this study, a total of 12 safflower genotypes, eight inbred lines and four commercial cultivars, were used. The names, flower color and spininess of these genotypes are indicated in Table 1. Callus and shoot formation were induced with 6-Benzylaminopurine [(BAP), (Sigma-Aldrich Product Number: B3408)] and 1-Naphthaleneacetic acid [(NAA), (Sigma-Aldrich Product Number: N0640) and also NAA was used for rooting of shoots. Surface sterilization of capitules was performed with mercuric chloride (PubChem CID: 24085).

Table 1. Flower colors and spininess of safflower genotypes used in anther culture study
Çizelge 1. Anter kültürü çalışmasında kullanılan aspir genotiplerinin çiçek renkleri ve dikenliliği

Numbers	Genotype names	Flower colors	Spininess
1	Balcı*	Yellow	Spiny
2	Dinçer*	Red	Spineless
3	58-11	Orange/ Red	Spineless
4	25	Yellow	Very few spines
5	37-5(3)	Orange	Spineless
6	38-4	Red	Spineless
7	Linas*	Red	Spiny
8	55-14	Orange/ Red	Spiny
9	Olas*	Orange	Spiny
10	43-11	Orange/ Red	Fewer spines
11	24	Yellow	Fewer spines
12	22	Red	Densely spines

*Commercial cultivars

The experimental design was performed in completely randomized plots involving three factors with four replications. The primary factor was safflower genotypes, the second factor was BAP (0, 0.5, 1, and 2 mg L^{-1}) doses, and the third factor was NAA (0, 0.1, and 0.5 mg L^{-1}) doses.

The application of anther culture in safflower genotypes was performed as described by Prasad et al. (1991). Immature capitules with white flowers, which indicates the early uninucleate stage of anther, were gathered from plants sowed in the field between 8 am and 9 am. First, the capitules were wrapped in aluminum foil and kept to cold treatment at +4ºC for 3-4 days. Then, capitules with removed bract leaves were sterilized with 0.1% mercuric chloride ($HgCl_2$) for 6 minutes and rinsed 5 times with sterile water. Following sterilization, flowers were transferred into sterile water to prevent drying. Anthers separated from flowers under a stereo microscope were transferred to MS media (Murashige & Skoog, 1962) consisting of varied doses of BAP and NAA.

At least 15 anthers for each hormone combination were cultured on 60 x 15 mm disposable petri dishes with full strength MS media consisting of 3% sugar for callus induction. Cultures were initially kept in the dark at 25ºC with 50-60% humidity for 10 days in Panasonic Climate Chambers (MLR-352H-PE) and then continued at the same culture condition with 16/8 day and night illumination. The rates of regenerable callus formation (RRCF) were determined 3-4 weeks after the beginning of culture. The calli were transferred to full strength MS medium involving 2% sugar in the same hormone combinations for shoot induction. Shoot formation was observed 3-4 weeks following the calli were incubated in the shooting medium. Then, developed shoots were moved to MS medium consisting of 0.5 mg L^{-1} of kinetin for shoot elongation during two weeks. Rooting was carried out

in a half-strength MS medium involving 0.1 mg L^{-1} of NAA with 1% sucrose.

The data were analyzed by using the JMP 14 statistical program. Arcsin √x transformation was applied to the % values that did not indicate the normal distribution and the mean values were compared according to the LSD and t-tests.

RESULTS and DISCUSSION

Regenerable callus formation

The high rate swelling in anthers of some safflower inbred lines such as 22, 24, 25, and 43-11 was observed compared to other genotypes in the first two weeks after the anther incubation, but compact and green calli were induced only in cvs. Balcı and Linas in the following two weeks (Figure 1a and Figure 1b). On the other hand, the calli of cv. Balcı indicated overgrowth (Figure 1c), it did not produce shoots as in cv. Linas (Figure 1d and Figure 1e). The shoots of cv. Linas were elongated with 0.5 mg L^{-1} of kinetin, but some of them had lost their vitality (Figure 1f).

The regenerable calli were induced in only cvs. Balcı and Linas among 12 safflower genotypes and also cv. Balcı indicated a higher RRCF than Linas (Table 2). These findings confirmed Lantos et al. (2022) who stated in vitro androgenesis is under genetic control in common crop plants. Prasad et al. (1991) and Thengane et al. (1994) also reported that anther response varied depending on safflower and sunflower genotypes, respectively.

High doses of BAP and NAA adversely affected the rate of regenerable callus (Table 2). The highest RRCF was obtained from 0.1 mg L^{-1} of NAA + 0.5 mg L^{-1} of BAP in cv. Balcı and 0.5 mg L^{-1} of NAA + 1 mg L^{-1} of BAP in cv. Linas (Table 3). All combinations of NAA with 2 mg L^{-1} of BAP did not produce regenerable callus in cv. Linas. Prasad et al. (1991) determined that the highest callus formation rates were obtained from combinations of 0.5, 1, 2, and 3 mg L^{-1} of BA and 0.5 mg L^{-1} of NAA in safflower, and the rate of callus formation was adversely affected by increasing NAA doses. On the other hand, some genus like *Dendranthema* (Khandakar et al., 2014), *Helianthus* (Voronova, 2016) and *Tagetes* (Kumar et al., 2018) belonging to the *Asteraceae* family indicated better responses to anther culture at 2 : 0.1, 2 : 1 and 2 : 0.5 mg L^{-1}, BAP : NAA), respectively.

Shoot regeneration

Although compact and green callus formation occurred at high rates in both cultivars, only 1 plant was obtained from the application of 1 mg L^{-1} of BAP + 0.1 mg L^{-1} of NAA in cv. Linas (Figure 1f). Unlike this finding, Prasad et al. (1991) obtained the highest shoot regeneration from anthers of safflower genotypes in a combination of 2 mg L^{-1} of BAP and 0.5 mg L^{-1} of NAA. This plantlet was rooted with 0.1 mg L^{-1} of NAA (Figure 1g), but it died because there was no root formation for a long time. Rootings initiated to 3-4 weeks after the death of shoot, but it did not turn green again (Figure 1h).

Table 2. Variance analysis and differences between mean values regarding the rate of regenerable callus formation by culturing anthers of some safflower genotypes at different BAP and NAA doses. (Mean ± standard error).

Çizelge 2. Bazı aspir genotiplerinde anterlerin farklı BAP ve NAA konsantrasyonlarında kültüre alınmasıyla oluşan rejenere olabilir kallus oranına ait varyans analizi ve ortalama değerler arasındaki farklar. (Ortalama ± standart hata).

Factors	Ratios of shoot-formingcallus (%)
Safflower genotypes	
Balcı	3.7±0.9[a]
Linas	1.9±0.8[b]
BAP doses (mg L^{-1})	
0	0.0±0.0[c]
0.5	4.8±1.6[a]
1	4.0±1.4[ab]
2	2.2±1.0[b]
NAA doses (mg L^{-1})	
0	0.5±0.3[b]
0.1	5.5±1.4[a]
0.5	2.2±1.0[b]
Analysis of variance	
Genotypes (A)	*
BAP doses (B)	**
NAA doses (C)	**
A × B	**
A × C	**
B × C	**
A × B × C	**

*, **: Significant level of 5% and 1%, respectively. †: Letters indicate different groups at the 5% level.

As a result of the callus and regeneration studies carried out on safflower genotypes, it was decided to continue the optimization of shooting and rooting with cv. Linas, which was sowed a second time in the late summer growing season. In this study, the highest RRCF was achieved at a combination of 0.5 mg L^{-1} of BAP and 0.1 mg L^{-1} of NAA with 20%, and seven shoots were derived from this combination. These shoots were moved to a medium consisting of 0.1 mg L^{-1} of NAA for rooting, but it did not occur. Belide et al. (2011) reported that safflower is sensitive to hyperhydration and difficult to genetically transform due to poor root formation in vitro.

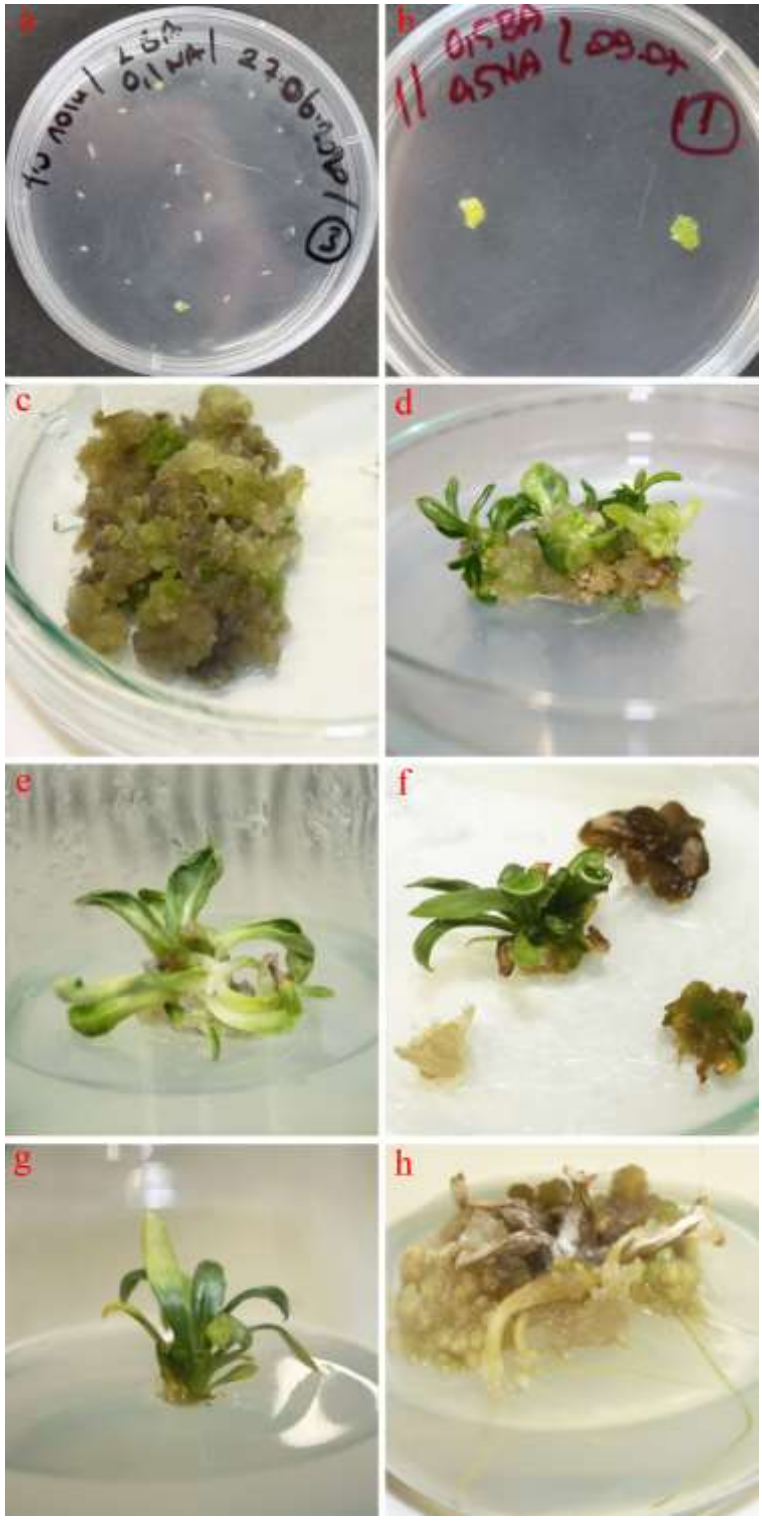

Figure 1. Shoot regeneration from anthers of safflower genotypes. 1a: Callus induction from anthers, 1b: Green and growing calli in cvs. Balcı and Linas, 1c: Callus that grew excessively but did not induce shoots in cv.Balcı, 1d and 1e: Vigorous and green calluses inducing shoots in cv. Linas, 1f: Shoots transferred for growth and elongation, 1g: Transferring of elongated shoots to rooting medium and 1h: Root development after shoot death.

Şekil 1. Aspir genotiplerinin anterlerinden sürgün rejenerasyonu. 1a: Anterlerden kallus oluşumu, 1b: Balcı ve Linas çeşitlerinde yeşil ve büyüyen kalluslar, 1c: Balcı çeşidinde aşırı büyüyen ancak sürgün vermeyen kallus, 1d ve 1e: Linas çeşidinde sürgün oluşturan güçlü ve yeşil kalluslar, 1f: Büyüme ve uzama için transfer edilen sürgünler 1g: Uzatılmış sürgünlerin köklenme ortamına aktarılması ve 1h: Sürgün ölümünden sonra kök gelişimi.

Table 3. The effect of varied BAP and NAA doses on the rate of regenerable callus formation in anthers of some safflower genotypes (%). (Mean ± standard error).

Çizelge 3. Farklı BAP ve NAA dozlarının bazı aspir genotiplerinin anterlerinde rejenere olabilir kallus oluşum oranına etkisi (%) (Ortalama ± standart hata).

Safflower genotypes	BAP doses (mg L^{-1})	NAA doses (mg L^{-1})		
		0	0.1	0.5
Balcı	0	0.0±0.0^{c*}	0.0±0.0^{c}	0.0±0.0^{c}
	0.5	0.0±0.0^{c}	13.3±0.0^{a}	6.7±3.8^{bc}
	1	0.0±0.0^{c}	11.1±2.2^{ab}	0.0±0.0^{c}
	2	2.3±2.2^{c}	11.1±2.2^{ab}	0.0±0.0^{c}
Linas	0	0.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{c}
	0.5	2.2±2.2^{c}	6.7±6.7^{bc}	0.0±0.0^{c}
	1	0.0±0.0^{c}	2.2±2.2^{c}	11.1±4.5^{ab}
	2	0.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{c}

* Letters indicate different groups at the 1% level for genotype × BAP × NAA interaction.

Table 4. Mean values of regenerable callus formation as a result of culturing anthers of cv. Linas in media containing different BA and NAA (%). (Mean ± standard error).

Çizelge 4. Linas çeşidine ait anterlerin farklı BA ve NAA içeren besi ortamlarında kültüre alınması sonucu oluşan rejenere olabilir kallus oranlarına ilişkin ortalama değerler (%). (Ortalama ± standart hata).

BAP doses (mg L^{-1})	NAA doses mg L^{-1}		
	0.1	0.5	Means
0.5	20.0±3.0^{a†}	1.3±1.3^{b}	10.7±4.9^{a}
1	0.0±0.0^{b}	2.7±1.6^{b}	1.4±1.3^{b}
2	6.7±4.2^{b}	0.0±0.0^{b}	3.4±3.2^{b}
Means	8.8±2.7^{a}	1.3±0.7^{b}	
Analysis of variance			
BAP doses (A)	*		
NAA doses (B)	**		
A × B	**		

*, **: Significant level of 5% and 1%, respectively. †: Letters indicate different groups at the 5% level.

CONCLUSION

In this study, the responses of 12 safflower genotypes to anther culture were determined. Among these genotypes, only cvs. Balcı and Linas produced highly regenerable callus. Although callus induction was observed in the other genotypes, these calli did not develop into compact and green calli, which have a higher potential to induce shoots. Among the genotypes producing regenerable callus, a few shoots were achieved only in cv. Linas. The elongation of these shoots was very important for rooting. However, the prolonged in vitro culture time to elongate of shoots caused the death of shoots due to hyperhydration. Rooting must be ensured within two weeks for the development of shoots. Although the shoots of cv. Linas were not initially rooted, the roots appeared after the shoots died due to hyperhydration by weeks 4 to 6. However, new shoots were not induced on these roots. If plenty of shoots would be obtained from the anther of safflower genotypes, new studies can be performed to increase rooting in different auxin combinations.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Author has declared no conflict of interest.

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