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Original scientific article

DEVELOPMENT OF EFFICIENT REGENERATION SYSTEMS FOR BARLEY CULTIVARS GROWN IN TÜRKİYE

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Successful use of modern plant breeding technologies relies on the availability of efficient regeneration systems. The present study was carried out to develop efficient regeneration systems in 18 barley cultivars grown in Türkiye, and to compare them with cultivar (cv.) Golden Promise, tissue culture standard cultivar in barley. Four different auxin types (dicamba, 2,4-D, NAA and IAA) were studied. Regeneration was carried out using 1 mg/L kinetin and 0.25 mg/L 2,4-D. Callus formation percentage, amount of callus produced and regeneration rate were different among cultivars, auxin types and cultivar x auxin combinations. Dicamba gave better results for callus formation percentage and amount of callus produced directly from embryos, while 2,4-D was better for amount of callus production in subcultures and regeneration rate. Compared to barley tissue culture standard cv. Golden Promise, cv. Angora had better callus production percentage, while cv. Çetin-2000 was better for amount of callus produced directly from embryos and cv. Tokak 157/37 was better for amount of callus produced in subculture. Regeneration rates of cultivars Cumhuriyet-50, Şerifehanım-98 and Çatalhöyük 2001 were not significantly different from that of cv. Golden Promise. Successful regeneration was achieved in other cultivars used except for cv. Orza. In conclusion, most Turkish cultivars had similar callus production frequency and amount similar to cv. Golden Promise, but lower plant regeneration rates.

Keywords: 2,4-D, Callus induction, Dicamba, Embryo culture, *Hordeum vulgare*, Naphthalene Acetic Acid

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INTRODUCTION

Transgenic technology allows introgression of useful genes from highly diverse sources into cultivated plants, thereby removing the barriers for plant breeders and accelerating the pace of crop improvement. However, use of transgenic technology depends on an efficient regeneration system in the involved crop species (HE *et al.,* 2023). In addition to transgenic technology, efficient regeneration systems have other uses in crop improvement such as shortening the time required to develop varieties through doubled haploid plants and embryo culture (DAHLEEN and BREGITZER, 2002). Barley has been extensively cultivated in Türkiye due to its significance as a primary source of livestock feed and a raw material in the malt industry. Embryo is most commonly used explant for plant regeneration in tissue culture of barley. Determining the regeneration capacity of embryo-originated calli in barley cultivars grown in Türkiye would be useful to establish an efficient regeneration system for biotechnology-based crop improvement.

Embryo culture is the most efficient method for regeneration in cereals. This method has been commonly used in direct gene transfer (MARTHE *et al.,* 2015). Besides other factors, genotype affects the success of embryo culture considerably (YIMAM *et al.,* 2025). In some studies conducted to improve the success of transgene introgression, genotype has been found the most significant factor affecting callus formation and plant regeneration (BENLIOGLU *et al.,* 2025). It has been found in another study that frequency of callus formation was affected by genotype both in mature and immature embryo culture (ABUMHADI *et al.,* 2005). Therefore, determining the callus formation and plant regeneration frequencies of cultivars are crucial for fast and efficient plant breeding programs.

Plant growth regulators play significant roles in culturing cereal embryos and plant regenerations from them. Auxin type growth regulators are needed to produce callus from embryos. Some important auxins are 2,4-D, Dicamba, Picloram, Naphthalene Acetic Acid (NAA) and Indole-3 Acetic Acid (IAA) (KUMAR *et al.,* 2017). SERHANTOVA *et al.* (2004) compared 2,4-D, dicamba and picloram for callus formation and plant regeneration, and found that media containing 2,4-D had the highest callus formation and plant regeneration rates. In another study with mature barley embryos, callus formation was studied using 2,4-D rates varying from 1 to 5 mg/L, and the highest callus production frequency was obtained in 3 mg/L 2,4-D application (HE and JIA, 2008). Using Harrington and Morex barley cultivars, BREGITZER *et al.* (1998) found that optimum 2,4-D concentration was genotype-specific, but 3 mg/L rate was found to be optimal for most cultivars.

Availability of an efficient callus production and plant regeneration system is crucial for efficient use of modern breeding technologies. Response of barley cultivars to tissue culture and optimum culture conditions for their regeneration capacity should be determined before an efficient use of such technologies. The aim of the present study was to determine the effects of different auxin types on callus production and plant regeneration capacity in some barley cultivars grown in Türkiye.

MATERIALS AND METHODS

Eighteen barley cultivars with commercial production in Türkiye along with cultivar (cv.) Golden Promise, which is considered a standard cultivar for tissue culture, were used in the present study (Table 1).

The study was conducted in Molecular Biotechnology and Tissue Culture laboratories and greenhouses of Tokat Gaziosmanpaşa University Faculty of Agriculture. Donor plants of embryo culture were grown in 18-22 °C temperature and 16 h light conditions during the day a 14-16 °C temperature and 8 h of dark during the night. Plants were grown in three-liter pots which contained ¼ part manure, ¼ part perlite, ¼ part peat and ¼ part soil mixture. Plants were fertilized with Hoagland nutrient solution (BREGITZER *et al.,* 1995). Spikes were harvested for embryo culture about 16-22 days after the fertilization when the seeds were plumpest but still green.

Table 1. Cultivars used in the study, their row type and origins

No	Cultivar name	Row type	Origin
1	Tokak 157/37	Two-row	Field Crops Central Research Institute
2	Cumhuriyet-50	Two-row	Anadolu Agric. Research Institute
3	Bülbül-89	Two-row	Field Crops Central Research Institute
4	Tarm-92	Two-row	Field Crops Central Research Institute
5	Efes 3	Two-row	Anadolu Brewing and Malt Industries
6	Yesevi-93	Two-row	Field Crops Central Research Institute
7	Orza-96	Two-row	Field Crops Central Research Institute
8	Kalaycı-97	Two-row	Transitional Zone Agric. Research Institute
9	Kıral-97	Six-row	Bahri Dagdas Int. Agric. Research Institute
10	Anadolu 98	Two-row	Anadolu Brewing and Malt Industries
11	Efes 98	Two-row	Anadolu Brewing and Malt Industries
12	Şerifehanım-98	Two-row	Aegean Agricultural Research Institute
13	Angora	Two-row	Anadolu Brewing and Malt Industries
14	Çetin-2000	Six-row	Field Crops Central Research Institute
15	Çumra 2001	Two-row	Anadolu Brewing and Malt Industries
16	Çatalhöyük 2001	Two-row	Anadolu Brewing and Malt Industries
17	Aydanhanım	Two-row	Field Crops Central Research Institute
18	Zeynelağa	Two-row	Field Crops Central Research Institute
19	Golden Promise	Two-row	The United Kingdom

The seeds were surface sterilized. For this aim, they were first treated with 70% ethyl alcohol for one min. followed by a 20% commercial bleach solution (about 1% final sodium hypochlorite concentration) with a few drops of Tween-20 for 15 min (BÜRÜN and POYRAZOĞLU, 2002) and rinsed with sterile water for three times. Culture medium consisted of a modified MS basal salt mixture (MURASHIGE and SKOOG, 1962) prepared by a commercial mixture (MURASHIGE and SKOOG Basal Salt Mixture, Sigma-Aldrich: M5524). Since $CuSO_4$ and H_3BO_3 were reported to promote callus production and regeneration (DAHLEEN and BREGITZER, 2002), $CuSO_4$ and H_3BO_3 were added to culture medium. Final composition of the culture medium was given in Table 2. pH was adjusted to 5.8. MS media for embryo culture were supplemented with four different auxin types (2,4-D, dicamba, NAA and IAA) at 3 mg/L rate (ZAPATA *et al.,* 2004). Each hormone type was replicated three times. Calli obtained from embryos directly or after subcultures were cultured on media containing 1 mg/L kinetin and 0.25 mg/L 2,4-D for shoot and root development.

After the surface sterilization, isolated embryos were cut along the embryonic axis to promote callus growth. Twenty half-embryos from 10 embryos were placed on a petri dish as the scutellum side upwards. Three weeks later, subculture was made using calli which developed from the cultured embryos. Ten callus pieces of about half a centimeter diameter were cultured on each petri dish during the sub-culturing. For callus development, cultures were kept at 25 ± 2 ºC temperature and dark conditions. For shoot regeneration, cultures were kept at 18-20 ºC day (16 h light) and 14-16 ºC night (8 h dark) conditions (MOKHTARI *et al.,* 2013).

Table 2. Modified Murashige and Skoog (MS) medium used

Ingredient	Amount
MS Salt	4.31 g/L
Sucrose	30 g/L
Thiamine	1 mg/L
Nicotinic acid	500 µg/L
Pyridoxine	500 µg/L
Myo-inositol	250 mg/L
Phytagel	3 g/L
$CuSO_4$	1.245 mg/L
H_3BO_3	40.15 mg/L

Developed shoots were transferred to Magenta trays which contained hormone-free MS medium for root development when they reached to a length of 2 cm. Seedlings of about 8-10 cm with well-developed roots were transferred to pots containing 2-3 cm thick, water-saturated peat. Top of the pots were covered with stretch film and water was sprayed on seedlings. Thus, seedlings were acclimatized for 3-4 days.

The following parameters were determined throughout the experiments:

Callus formation rate (%): Percentage of explants which produced callus 20 days after the initiation of culture was determined.

Average amount of callus production per embryo (mg): Total weight of calli from callus producing embryos were divided by the number of callus producing embryos.

Callus production amount per callus after sub-culture (mg): Total weight of callus pieces placed on subculture was subtracted from the total weight of calli developed in subculture.

Regeneration rate (%): Percentage of plantlet-producing callus obtained directly from embryos was calculated.

Regeneration rate after sub-culturing (%): Percentage of plantlet-producing callus grown in subculture was calculated.

The experimental design was completely randomized design arranged in factorial pattern. MSTAT-C statistical software was used in all statistical analyses (FREED and

EISENSMITH, 1986). Percent values were subjected to arc sin transformation before the statistical analyses. Differences among the means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Callus formation rate

Differences between barley cultivars and auxin types, and cultivar x auxin type interactions were significant ($P<0.01$) for callus formation rate (Table 3). Although four different auxin types were used in the study, IAA did not produce any callus in any cultivars, and therefore it was removed from statistical analyses. As the average of cultivars, Dicamba produced callus in 96.47% of the explants while 2,4-D produced callus in 86.74% and NAA in 32.32%. Higher callus formation rate by Dicamba was also reported by HALAMKOVA *et al.* (2004). Because of its low callus production rate, NAA is not suitable for an efficient callus production. Similarly, ZIAUDDIN and KASHA (1990) reported that callus production percentage of NAA was lower than that of 2,4-D. High doses of IAA and NAA (5-50 mg/L) are required for a satisfactory level of callus production rate. However, since high auxin levels could hinder subsequent plant regeneration (MATHEW *et al.*, 2024) and lead to somatic mutations (MAJUMDER *et al.*, 2025), it may not be appropriate to use high auxin levels when sub-culturing is needed.

Table 3. Effect of different auxin types on callus formation percentage of different barley cultivars

Cultivars	2,4-D	Dicamba	NAA	Mean
Tokak 157/37	96.67 AB**	100.00 A	30.48 H	75.71 AB**
Cumhuriyet-50	82.63 B-E	100.00 A	51.17 FGH	77.93 ABC
Bülbül-89	100.00 A	100.00 A	32.93 H	77.64 A
Tarm-92	90.83 A-E	91.67 A-D	34.03 H	72.18 BCD
Efes 3	85.97 A-E	98.33 AB	26.32 H	70.21 BCD
Yesevi-93	86.67 B-E	98.33 AB	30.93 H	71.98 BCD
Orza-96	50.00 FGH	90.00 A-E	30.74 H	56.91 E
Kalaycı-97	66.67 EFG	75.00 DEF	28.43 H	56.70 E
Kıral-97	98.33 AB	100.00 A	0.00 I	66.11 D
Anadolu 98	80.00 CDE	96.67 AB	37.04 GH	71.23 BCD
Efes 98	73.90 DEF	100.00 A	30.53 H	68.14 BCD
Şerifehanım-98	98.23 AB	100.00 A	28.70 H	75.64 AB
Angora	98.13 AB	100.00 A	43.60 GH	80.59 A
Çetin-2000	81.67 B-E	98.33 AB	37.97 GH	72.66 BCD
Çumra 2001	80.00 B-E	100.00 A	28.07 H	69.36 BCD
Çatalhöyük 2001	98.33 AB	98.15 AB	36.78 GH	77.76 AB
Aydanhanım	93.33 ABC	95.00 ABC	35.22 H	74.51 A-D
Zeynelağa	96.67 AB	98.15 AB	40.16 GH	78.32 AB
Golden Promise	90.00 A-E	93.25 A-D	30.90 H	71.38 CD
Mean	86.74 B**	96.47 A	32.32 C	

**: Significant at $p<0.01$. Differences between the means with the same letter are not statistically significant. NAA: Naphthalene Acetic Acid

Angora had the highest percentage of callus formation (80.59%). Except for cv. Orza-96, callus formation rate was 66% and over in all cultivars used. It was striking that six of the 18 cultivars had significantly higher callus production percentage than cv. Golden Promise, standard barley cultivar for tissue culture (MARTHE *et al.*, 2015; XU *et al.,* 2022).

Significant differences were observed among the cultivars for their responses to different growth regulator types. Dicamba auxin produced callus formation rates of over 90% in all cultivars except for cv. Kalaycı-97. 2,4-D auxin, on the other hand, produced callus by 90% or over in 10 of 19 cultivars used. The lowest callus formation rate was observed in cv. Orza-96 by 50%, followed by cultivars (cvs.) Kalaycı-97 and Efes 98. Other cultivars produced callus with a callus formation rate of 80% and over. NAA auxin did not produce callus in cv. Kıral-97, while cv. Cumhuriyet-50 had the highest callus production percentage (51.17%) with this auxin. Cv. Orza-96 had almost twice higher callus production percentage with dicamba compared to with 2,4-D. These two auxins resulted in similar callus formation rates in cvs. Tokak 157/37, Bülbül-89, Tarm-92, Kıral-97, Şerifehanım-98, Angora, Çatalhöyük 2001, Aydanhanım, Zeynelağa and Golden Promise. Genotype was found to be the most significant factor affecting callus formation rate in similar studies (YIMAM *et al.*, 2025; ZHANG *et al.*, 2022). Thus, different callus formation rates of cultivars with different auxin types could make it necessary to use the most suitable auxin for different cultivars.

Amount of callus production

In terms of amount of callus produced from embryo or from sub-cultured callus, effects of cultivars and auxin types, as well as their interactions, were significant ($p < 0.01$). Dicamba produced highest amount of callus directly from embryos while 2,4-D produced highest amount of callus from subcultures (Table 4). Efficiency of dicamba auxin to produce callus from embryo was also reported by SATYAVATHI *et al.* (2004) and KUMAR *et al.* (2017). NAA produced least amount of callus both directly from embryos and in subcultures.

Amount of callus produced directly from embryos was highest in cv. Çetin-2000, and lowest in cv. Kalaycı-97. Cultivars other than Tokak 157/37, Orza-96, Çetin-2000 and Çumra 2001 were not significantly different from cv. Golden Promise for callus production from embryos. On the other hand, amount of callus in subcultures was highest in Tokak 157/37. Cvs. Cumhuriyet-50, Bülbül-89, Tarm-92, Efes 3, Anadolu 98 and Çumra 2001 had similar callus production in subcultures to that of Golden Promise, while other cultivars produced less in subcultures. Thus, Tokak 157/37 produced higher amount of callus than cv. Golden Promise both directly from embryos and in subcultures.

Callus productions directly from embryos or in subculture were higher in dicamba auxin in cvs. Tokak 157/37, Bülbül-89, Orza-96 and Anadolu 98, while cvs. Cumhuriyet-50, Angora and Aydanhanım had better results for these productions in 2,4-D auxin. Callus productions from embryos were not considerably different in cv. Golden Promise compared to other cultivars when NAA was used. The same results were observed for callus production in subcultures. However, some cultivars gave better response to dicamba auxin. Similarly, ERKOYUNCU and YORGANCILAR (2016) reported that cv. Bülbül-89 produced higher amount of callus than cv. Tokak 157/37 in dicamba. Callus production of cv. Golden Promise was higher with dicamba in direct callus production from embryos, while 2,4-D was better for callus production in

subcultures. It could be stated that 2,4-D was more efficient for callus production in subcultures compared to callus production directly from embryos.

Table 4. Effect of different auxin types on amount of callus production (mg) directly from embryo and in subculture

	From embryo				In subculture			
Cultivars	2,4-D	Dicamba	NAA	Mean	2,4-D	Dicamba	NAA	Mean
Tokak 157/37	75.80 e-o**	147.50 a	39.17 m-s	87.48 ab**	105.30 a-h**	116.50 a-d	98.62 a-j	106.9 a**
Cumhuriyet-50	75.75 e-o	60.10 j-q	48.21 m-r	61.35 cde	117.80 a-d	69.85 f-n	77.38 b-n	88.30 bc
Bülbül-89	73.13 f-p	126.20 a-d	28.30 o-s	75.88 abc	104.00 a-i	130.00 a	58.44 j-q	97.50 ab
Tarm-92	101.30 b-k	98.25 c-k	32.85 n-s	77.47 abc	85.30 b-l	96.18 a-j	75.39 d-n	85.60 bc
Efes 3	84.27 d-m	108.90 a-i	27.84 p-s	73.68 bc	110.80 a-g	109.10 a-g	68.45 g-o	96.10 ab
Yesevi-93	66.03 i-q	96.37 c-l	62.90 i-q	75.10 abc	111.50 a-g	69.93 f-n	26.42 o-r	69.20 de
Orza-96	55.07 k-r	134.60 abc	49.56 l-r	79.75 ab	51.33 k-q	111.10 a-f	37.90 n-r	67.10 de
Kalaycı-97	44.35 m-s	66.24 i-q	31.89 n-s	47.49 e	48.64 l-q	39.71 m-r	18.29 qr	35.50 f
Kıral-97	77.24 e-n	106.40 a-j	0.00 s	61.20 cde	86.67 b-l	84.75 b-l	0.00 r	57.10 e
Anadolu 98	78.57 e-n	144.50 ab	8.97 rs	77.35 abc	90.76 a-l	113.90 a-e	63.88 h-p	89.50 bc
Efes 98	72.56 f-q	115.60 a-h	46.72 m-r	78.29 abc	101.10 a-j	75.89 d-n	26.73 o-r	67.90 de
Şerifehanım-98	58.64 k-q	64.50 i-q	47.59 m-r	56.91 de	65.14 h-p	61.70 i-p	69.79 f-n	65.50 de
Angora	99.95 b-k	69.89 h-q	46.24 m-r	72.03 bcd	114.10 a-e	50.54 k-q	62.75 h-p	75.80 cd
Çetin-2000	118.30 a-f	117.07 a-g	39.65 m-s	91.66 a	59.55 j-q	83.46 b-l	22.50 pqr	55.20 e
Çumra 2001	83.68 d-m	124.10 a-d	54.63 k-r	87.48 ab	120.20 ab	93.78 a-k	59.19 j-q	91.10 b
Çatalhöyük 2001	56.40 k-q	119.20 a-e	44.34 m-s	73.30 bcd	73.27 e-n	78.17 b-n	50.24 k-q	67.20 de
Aydanhanım	71.90 g-q	47.00 m-r	43.05 m-s	53.98 e	119.20 abc	48.80 l-q	19.53 qr	62.50 de
Zeynelağa	72.40 f-q	72.61 f-q	25.30 qrs	56.77 de	83.53 b-l	89.58 a-l	50.93 k-q	74.70 cd
Golden Promise	49.23 l-r	107.40 a-i	28.53 o-s	61.71 cde	116.30 a-e	82.42 b-m	76.18 c-n	91.60 b
Mean	74.42 b**	101.40 a	37.19 c		92.84 a**	84.51 b	50.65 c	

**: Significant at p<0.01. Differences between the means with the same letter are not statistically significant. NAA: Naphthalene Acetic Acid

Plant Regeneration rate

Differences among cultivars and auxin types, as well as their interaction, were significant for plant regeneration rate from callus ($p < 0.01$). The highest regeneration rate was obtained from calli produced by 2,4-D both directly from embryos and in subcultures, followed by dicamba and NAA (Table 5). Higher regeneration rates in calli produced by 2,4-D were also reported in previous studies (CHANG *et al.*, 2003; SERHANTOVA *et al.*, 2004). Regeneration rate was lower in calli directly produced from embryos compared to those produced in subcultures. Similar results were found by BREGITZER *et al.* (1995) and CHANG *et al.* (2003).

Plant regeneration rates of the cultivars varied from 0 to 31.29%. The highest regeneration rate was obtained from cv. Golden Promise both in calli directly produced from embryos and those produced in subcultures. Regeneration rates of cvs. Cumhuriyet-50, Tarm-92, Şerifehanım-98 and Çatalhöyük 2001 in calli directly produced from embryos were not

significantly different from those of cv. Golden Promise, while those of Cumhuriyet-50, Şerifehanım-98 and Çatalhöyük 2001 were not different from cv. Golden Promise in calli grown in subcultures. Other cultivars had lower plant regeneration values than cv. Golden Promise. Cv. Orza-96 did not regenerate any plant in any condition. It seemed that superiority of cv. Golden Promise in tissue culture comes from its high regeneration capability. Regeneration rate of cv. Golden Promise was 42% during the eight week of cultures (BREGITZER, 1992) and 55% during the eleven weeks of cultures (SHARMA *et al.,* 2005).

In terms of cultivar x auxin combinations, the highest regeneration rate in calli grown directly from embryos was achieved from cv. Tarm-92 calli produced by 2,4-D auxin. 2,4-D produced similar regeneration rates in all cultivars except for cvs. Orza-96, Kalaycı-97 Kıral-97 and Çetin-2000. In calli produced by dicamba, regeneration rate was highest in cvs. Tarm-92 and Golden Promise, but regeneration rates of these two cultivars were not different from the 10 cultivars following them. Regeneration was obtained in calli produced by NAA in only eight cultivars, and five of them had similar rates as cv. Golden Promise. NAA resulted in similar regeneration rates to other auxins in cv. Golden Promise.

Table 5. Effects of different auxin types on plant regeneration rate of barley cultivars (%)

	Calli produced from embryos				Calli produced in subcultures			
Cultivars	2,4-D	Dicamba	NAA	Mean	2,4-D	Dicamba	NAA	Mean
Tokak 157/37	34.10 a-d**	5.53 e-h	0.00 h	13.21 bcd**	13.89 a-e**	2.78 de	0.00 e	5.56 b-e**
Cumhuriyet-50	31.33 a-e	16.67 a-h	18.00 a-g	22.00 ab	16.67 a-d	15.00 a-d	0.00 e	10.56 abc
Bülbül-89	27.77 a-e	5.53 e-h	14.00 a-h	15.77 bc	13.89 a-d	2.78 de	0.00 e	5.56 b-e
Tarm-92	41.67 a	36.10 abc	0.00 h	25.92 ab	11.82 a-e	11.11 a-e	0.00 e	7.64 bcd
Efes 3	19.47 a-g	8.33 c-h	12.33 b-h	13.38 bc	11.11 a-e	2.78 de	0.00 e	4.63 b-e
Yesevi-93	13.90 a-h	8.30 b-h	0.00 h	7.40 cde	8.33 a-e	3.70 b-e	0.00 e	4.01 b-e
Orza-96	0.00 h	0.00 h	0.00 h	0.00 e	0.00 e	0.00 e	0.00 e	0.00 e
Kalaycı-97	8.33 d-h	0.00 h	0.00 h	2.78 de	6.48 a-e	0.00 e	0.00 e	2.16 de
Kıral-97	8.33 e-h	3.70 fgh	0.00 h	4.01 de	5.81 a-e	3.33 cde	0.00 e	3.05 b-e
Anadolu 98	11.10 a-h	0.00 h	0.00 h	3.70 de	8.93 a-e	0.00 e	0.00 e	2.98 cde
Efes 98	16.67 a-h	0.00 h	11.33 b-h	9.33 cde	9.26 a-e	0.00 e	12.22 a-e	7.16 b-e
Şerifehanım-98	33.30 a-d	30.53 a-e	0.00 h	21.28 abc	11.11 a-e	18.97 abc	0.00 e	10.03 abc
Angora	25.00 a-f	9.90 c-h	4.67 fgh	13.19 bcd	8.12 a-e	2.78 de	0.00 e	3.63 b-e
Çetin-2000	2.77 gh	0.00 h	0.00 h	0.92 e	0.00 e	0.00 e	0.00 e	0.00 e
Çumra 2001	22.23 a-g	11.10 a-h	0.00 h	11.11 bcd	5.55 a-e	0.00 e	0.00 e	1.85 de
Çatalhöyük 2001	38.87 ab	11.13 b-h	24.67 a-f	24.89 ab	13.89 a-d	6.11 a-e	10.32 a-e	10.11 ab
Aydanhanım	22.23 a-g	25.03 a-f	0.00 h	15.76 bc	8.33 a-e	14.14 a-d	0.00 e	7.49 bcd
Zeynelağa	25.03 a-f	5.53 e-h	3.67 fgh	11.41 bcd	11.24 a-e	0.00 e	4.17 a-e	5.13 b-e
Golden Promise	33.30 a-d	30.57 a-e	30.00 a-e	31.29 a	18.79 ab	19.45 a	15.00 a-e	17.74 a
Mean	21.86 a**	10.95 b	6.25 c		9.64 a**	5.42 b	2.20 c	

**: Significant at p<0.01. Differences between the means with the same letter are not statistically significant. NAA: Naphthalene Acetic Acid

The highest regeneration rate in subcultures was obtained from cv. Golden Promise calli produced by dicamba auxin. Calli grown in subcultures of Orza-96 and Çetin-2000 cultivars produced using 2,4-D auxin did not regenerate any plants. However, other cultivars had regeneration rates not significantly different from that of cv. Golden Promise from calli grown in subculture using this auxin. No regeneration was obtained in callus subcultures produced from dicamba auxin in seven cultivars while cvs. Cumhuriyet-50 Tarm-92, Şerifehanım-98, Çatalhöyük 2001 and Aydanhanım had regeneration rates similar to cv. Golden Promise. NAA auxin produced callus in cvs. Efes 98, Çatalhöyük 2001, Zeynelağa and Golden Promise, while other cultivars did not produced callus in response to NAA. In general, regeneration rate was lower in calli produced in subcultures. Lower regeneration rates in calli produced in subcultures were also reported by CHANG *et al.* (2003). Besides cv. Golden Promise, Çatalhöyük 2001 was the only cultivar which produced callus in the presence of all three auxins.

The plant regeneration rate is the major parameter for the success in tissue culture. In the present study, some cultivars performed better than Golden Promise in terms of callus formation rate, amount of callus production from embryos and subcultures, and plant regeneration rate of calli produced from embryos in different hormones. However, Golden Promise was better than all other cultivars in terms of plant regeneration rate in subcultures in all hormones. The superior tissue culture response of Golden Promise compared to other cultivars was related to its high plant regeneration rate even after the subcultures. This can be explained by internal hormonal composition of Golden Promise, which is quite suitable for tissue culture. HISANO *et al.* (2016) reported that levels of plant hormones affecting shoot and root development (i.e., plant regeneration) were higher in Golden Promise than in recalcitrant cultivars. WANG *et al.* (2023) concluded that Golden Promise was more efficient in genetic transformation because the expression of genes associated with auxin and cytokinin is higher in almost all stages of callus multiplication. In addition, studies to reveal the reasons for tissue culture success of Golden Promise found that Golden Promise has higher levels of certain metabolites in tissue culture (HE *et al.,* 2023) and higher expression levels of certain genes (XU *et al.,* 2022) than recalcitrant cultivars. Besides, Golden Promise was reported to carry three important genomic regions that influence tissue culture success (HISANO and SATO, 2016).

CONCLUSIONS

Genotype and hormone type are the major factors determining the response in embryo cultures. Choice of the appropriate growth medium for recalcitrant cultivars is crucial in regeneration of these cultivars. For the embryo culture of barley cultivars grown in Türkiye, dicamba auxin seemed better for callus formation rate and amount of callus production directly from embryos, while 2,4-D produced higher amount of callus in subculture and, more importantly, had higher plant regeneration rates in calli both produced directly from embryos and in subcultures. Except for cv. Orza-96, suitable regeneration was achieved for cultivars used in the present study. Although amount of callus production in subculture was higher in cv. Tokak 157/37 than cv. Golden Promise, plant regeneration rates of cv. Golden Promise was better in calli produced both directly from embryos and in subcultures.

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REFERENCES

ABUMHADI, N., K., KAMENAROVA, E., TODOROVSKA, G., DIMOV, A., TRIFONOVA, K., GECHEFF, A, ATANASSOV (2005): Callus induction and plant regeneration from barley mature embryos (*Hordeum vulgare* L.*)*. Biotechnol. Biotechnol. Equip., *19*(3): 32-38.

BENLIOGLU, B., A., TÜRKOĞLU, M., INCETEKIN, H., OZAKTAN, C., DEMIR, G., AKDOGAN, J., BOCIANOWSKI (2025): Evaluation of genetic diversity of two-row barley by tissue culture potential. Genet. Resour. Crop. Evol., 1-14.

BREGITZER, P. (1992): Plant regeneration and callus type in barley: effect of genotype and culture medium. Crop Sci., *32*(5): 1108-1112.

BREGITZER, P., R.D., CAMPBELL, Y., WU (1995): Plant regeneration from barley callus: effects of 2,4-dichlorophenoxyacetic acid and phenylacetic acid. Plant Cell Tiss. Org., *43*(3): 229-235.

BREGITZER, P., L.S., DAHLEEN, R.D., CAMPEBELL (1998): Enhancement of plant regeneration from embryogenic callus of commercial barley cultivars. Plant Cell Rep., *17*(12): 941-945.

BÜRÜN, B., E.C., POYRAZOĞLU (2002): Embryo culture in barley (*Hordeum vulgare* L.). Turk. J. Biol., *26*(3): 175-180.

CHANG, Y., J.V., ZITZEWITZ, P.M., HAYES, T.H.H., CHEN (2003): High frequency plant regeneration from immature embryos of an elite barley cultivar (*Hordeum vulgare* L.cv. Morex*).* Plant Cell Rep., *21*(8): 733-738.

DAHLEEN, L.S., P., BREGITZER (2002): An improved media system for high regeneration rates from barley immature embryo-derived callus cultures of commercial cultivars. Crop Sci., *42(*3): 934-938.

ERKOYUNCU, M.T., M., YORGANCILAR (2016): Efficient callus induction and plant regeneration from mature embryo culture of barley (*Hordeum vulgare* L.) genotypes. International Journal of Agricultural and Biosystems Engineering *10*(6): 347-353.

FREED, R.D., S.P., EISENSMITH (1986): MSTAT microcomputer statistical program. Michigan State University of Agriculture and Applied Science, Michigan, USA.

HALAMKOVA, E., J., VAGERA, L., OHNOUTKOVA (2004): Regeneration capacity of calli derived from immature embryos in spring barley cultivars. Biol. Plantarum, *48*(2): 313-316.

HE, X., Z., GU, G., ZHANG, L., YE (2023): Identification of metabolites associated with plant regeneration capacity of barley callus. Plant Growth Regul., *100*(1): 71-83.

HE, T., J.F., JIA (2008): High frequency plant regeneration from mature embryo explants of highland barley (*Hordeum vulgare* L. var. nudum Hk. f.) under endosperm – supported culture. Plant Cell Tiss. Org., 95(2): 251-254.

HISANO, H., K. SATO (2016): Genomic regions responsible for amenability to Agrobacterium-mediated transformation in barley. Sci. Rep., *6*(1): 37505.

HISANO, H., T., MATSUURA, I.C., MORI, M., YAMANE, K., SATO (2016): Endogenous hormone levels affect the regeneration ability of callus derived from different organs in barley. Plant Physiol. Biochem., *99*: 66-72.

KUMAR, R., H.M., MAMRUTHA, A., KAUR, K., VENKATESH, A., GREWAL, R., KUMAR, V., TIWARI (2017): Development of an efficient and reproducible regeneration system in wheat (*Triticum aestivum* L.). Physiol, Mol. Biol. Plants, *23*(4): 945-954.

MAJUMDER, S., A.U., IGAMBERDIEV, S.C.I DEBNATH (2025): Somaclonal Variation and Clonal Fidelity in Commercial Micropropagation: Challenges and Perspectives. Agronomy, 15(6): 1489.

MARTHE, C., J., KUMLEHN, G., HENSEL (2015): Barley (*Hordeum vulgare* L.) transformation using immature embryos. In: Wang K., (Ed), Agrobacterium Protocols, Springer, New York, USA.

MATHEW, M. M., A., GANGULY & K.PRASAD (2024:). Multiple feedbacks on self-organized morphogenesis during plant regeneration. New Phytologist, *241*(2): 553-559.

MOKHTARI, A., H., ALIZADEH, B.Y., SAMADI, M., OMIDI, M., OTROSHY, Z., MOEINI (2013): Effect of plant growth regulators on direct shoot regeneration of wheat immature embryonic explants. Int. J. Agr. Biol. Eng., *1*(3): 74-80.

MURASHIGE, T., F., SKOOG (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, *15*(3): 473-497.

SATYAVATHI, V.V., P.P., JAUHAR, E.M., ELIAS, M.B., RAO (2004): Effects of growth regulators on in vitro plant regeneration in durum wheat. Crop Sci., *44*(5): 1839-1846.

SERHANTOVA, V., J., EHRENBERGEROVA, L., OHNOUTKOVA (2004): Callus induction and regeneration efficiency of spring barley cultivars registered in the Czech Republic. Plant Soil Environ., *50*(10): 456-462.

SHARMA, V.K., R., HANSCH, R.R., MENDEL, J., SCHULZE (2005): Mature embryo axis-based high frequency somatic embryogenesis and plant regeneration from multiple cultivars of barley (*Hordeum vulgare*). J. Exp. Bot., *56*(417): 1913-1922.

WANG, F., B., HE, Y., HONG, L., FU, Q., SHEN, G., ZHANG (2023): Time-course of genotype and hormone-related effects on callus proliferation in barley genetic transformation. Crop Design, *2*(2): 100043.

XU, Z., F., WANG, Y., TU, Y., XU, Q., SHEN, G., ZHANG (2022): Transcriptome analysis reveals genetic factors related to callus induction in barley. Agronomy, *12*(3): 749.

YIMAM, T., M., ABIDE, S., BENOR, D., GUADIE (2025): Protocol optimization for callus induction and shoot regeneration of Ethiopian rice varieties (Oryza sativa L.). BMC Biotechnol., *25*: 43.

ZAPATA, J.M., M., SABATER, M., MARTIN (2004): Callus induction and in vitro regeneration from barley mature embryos. Biol. Plantarum., *48*(3): 473-476.

ZHANG, K., D., DU, W. WANG (2022): Transcriptome Profiling of Different State Callus Induced from Immature Embryo in Maize. Journal of Chem., 6237298.

ZIAUDDIN, A., K.J., KASHA (1990): Long-term callus cultures of diploid barley (*Hordeum vulgare*) I. Auxin effects on culture initiation and maintenance. Euphytica *48*(2): 171–176.

RAZVOJ EFIKASNOG SISTEMA ZA REGENERACIJU SORTI JEČMA U TURSKOJ

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Izvod

Uspešna upotreba modernih tehnologija oplemenjivanja biljaka oslanja se na dostupnost efikasnih sistema regeneracije. Ova studija je sprovedena radi razvoja efikasnih sistema regeneracije kod 18 sorti ječma uzgajanih u Turskoj i njihovog upoređivanja sa sortom (cv.) Golden Promise, standardnom sortom ječma za kulturu tkiva. Proučavana su četiri različita tipa auksina (dikamba, 2,4-D, NAA i IAA). Regeneracija je sprovedena korišćenjem 1 mg/L kinetina i 0,25 mg/L 2,4-D. Procenat formiranja kalusa, količina proizvedenog kalusa i brzina regeneracije razlikovali su se među sortama, tipovima auksina i kombinacijama sorta x auksin. Dikamba je dala bolje rezultate za procenat formiranja kalusa i količinu kalusa proizvedenog direktno iz embriona, dok je 2,4-D bio bolji za količinu proizvedenih kalusa u subkulturama i brzinu regeneracije. U poređenju sa standardnom kulturom tkiva ječma, cv. Golden Promise, cv. Angora je imala bolji procenat proizvodnje kalusa, dok je cv. Sorta Çetin-2000 je bila bolja u pogledu količine kalusa proizvedenog direktno iz embriona, a sorta Tokak 157/37 je bila bolja u pogledu količine kalusa proizvedenog u subkulturi. Stope regeneracije sorti Cumhuriyet-50, Şerifehanım-98 i Çatalhöyük 2001 nisu se značajno razlikovale od stopa kod sorte Golden Promise. Uspešna regeneracija je postignuta i kod drugih korišćenih sorti, osim kod sorte Orza. Zaključno, većina turskih sorti imala je sličnu učestalost proizvodnje kalusa i količinu kao i sorta Golden Promise, ali niže stope regeneracije biljaka.

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