World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:10, No:6, 2016

Efficient Callus Induction and Plant Regeneration from Mature Embryo Culture of Barley (*Hordeum vulgare* L.) Genotypes

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***Abstract*—**Crop improvement through genetic engineering depends on effective and reproducible plant regeneration systems. Immature embryos are the most widely used explant source for *in vitro* regeneration in barley (*Hordeum vulgare* L.). However, immature embryos require the continuous growth of donor plants and the suitable stage for their culture is also certainly limited. On the other hand, mature embryos can be procured and stored easily; they can be studied throughout the year. In this study, an effective callus induction and plant regeneration were aimed to develop from mature embryos of different barley genotypes. The effect of medium (MS_1 and MS_2), auxin type (2,4-D, dicamba, picloram and 2,4,5-T) and concentrations (2, 4, 6 mg/l) on callus formation and effect of cytokinin type (TDZ, BAP) and concentrations (0.2, 0.5, 1.0 mg/l) on green plant regeneration were evaluated in mature embryo culture of barley. Callus and shoot formation was successful for all genotypes. By depending on genotype, MS_1 is the best medium, 4 mg/l dicamba is the best growth regulator in the callus induction and MS_1 is the best medium, 1 mg/l BAP is the best growth regulator in the shoot formation were determined.

***Keywords*—**Barley, callus, embryo culture, mature embryo.

I. Introduction

GENETIC studies done by gene transfer methods and other biotechnical developments in cereals are based on creating an effective and repeatable plant regeneration system. However, dependency on genotype, low regeneration frequency, albinism and low fertility rate in regenerative plants are still significant problems in plant regeneration systems [1]

One of the tissue culture applications used in crop improvement is embryo culture applications. Plant regeneration systems can be created with effective and repeatable embryo culture methods. Thus, gene transfer can be performed through *Agrobacterium tumefaciens* or by particle bombardment to the cultured plant cells or tissues; then, transgenic plants carrying the transferred gene can be obtained.

In most of the embryo culture studies done in the previous years, matured embryo in recalcitrant plants such as monocot species are reported as the best explant sources when evaluated in terms of regeneration [2]. Nowadays, explants used for plant regeneration are re-evaluated and studies are carried out with mature embryo [1], [3]. The use of mature embryo offers remarkable advantages in comparison to the use of immature embryo. Thus, there is no need to grow donor plants in greenhouses under controlled environmental conditions that require intense labor, time and space. The need for vernalization, especially in winter species leads to extra loss of time. Also, the desired amounts of seeds can be obtained throughout the year and the effect of environmental factors on tissue culture is eliminated [4]. Because of all these reasons, mature embryo is considered as the advantageous explant source of cereal tissue culture investigations. However, low regeneration frequency of mature embryo constitutes a major obstacle. Mechanical and chemical *in vitro* techniques applied to mature embryo [1], the endosperm-supported callus induction method [5], [6] are successfully used to improve regeneration frequency.

This research was conducted with the purpose of creating an effective callus from mature embryo of three barley genotypes and developing a plant regeneration system

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II. Material And Methods

In this study, Karatay-94, Bülbül-89 genotypes and F_2 generation seeds of Karatay-94xBülbül-89 hybrid obtained by Selcuk University Faculty of Agriculture, Department of Field Crops were used as plant material.

After the seeds were pre-sterilized for 2 minutes in ethanol of 96%, they were left in 20% sodium hypochlorite (NaOCI) solution including a few drops of Tween 20 (Sigma) for 20 minutes by being stirred and then, rinsed three times with a sterile distilled water. The sterile seeds were kept in 4 °C sterile water for 2 days after sterilization in order to separate embryo from the seed without damaging by softening the testa. Scutellum of removed embryo with the help of pliers on sterile tiles and in a sterile cabinet were cultured as upwards in 100 x 20 mm petri dishes which have 30 ml nutrient medium and there are 10 embryos in each petri containing 3 replicates petri dishes and each replicate has 3 petri dishes.

In the experiment done with the purpose of determining appropriate growth regulator (auxins) for callus induction, mature embryo was cultured in medium called as MS_1 (containing MS mineral salts, 200 mg/l myo-inositol, 0.5 mg/l Thiamine, 500 mg/l casein hydrolyzate, 100 mg/l glutamine, 250 g/l proline, 30 mg/l sucrose, 8 gr/l agar) and MS_2 (containing MS mineral salts, 200 mg/l myo-inositol, 1 mg/l Thiamine, 1000 mg/l casein hydrolyzate, 500 g/l proline, 60 mg/l maltose, 8 gr/l agar) by being modified from MS [7]

media including 2.4-D, dicamba, picloram, and 2.4.5-T auxins separately in 2 mg/l concentration. Their development was followed in growth cabinet under 24±2 °C, in the dark for 4 weeks; afterwards, the studies were continued in both media (MS_1 and MS_2) in three different concentrations (2, 4, 6 mg/l) of two different auxins (dicamba and 2.4-D) that provided the best results. Approximately 10 days after the culturing process, shoots and roots formed in embryo that showed germination as well as callus formation were removed; therefore, callus formation was accelerated.

Calli in the callus induction media were transferred to MS_1 media that contain each cytokine separately in (0.2-0.5 mg/l) TDZ and (0.5-1 mg/l) BAP concentrations at the end of 4 weeks. They were cultured in growth cabinet (Sanyo: MLR-351H) under 24 ± 2°C temperature, 65% humidity, 5 LS light intensity for 4 weeks in a way that it is 16/8 photoperiod time. The all media were adjusted to pH 5.8 and autoclaved for 20 min at 121°C and 1.1 kg/cm^2 pressure.

In this study, callus formation percentage (%), callus weight (mg/explant), shoot formation percentage (%) observations were obtained. All experiments were arranged according to factorial experiment designs in coincidence parcels and the acquired data were analyzed by using MSTAT-C statistical program. Significant differences were compared by LDS multiple comparison test.

III. Results and Discussion

A. Determination of the Auxin Type

With the purpose of determining suitable auxin type that will provide callus induction in three different barley genotypes, embryos were isolated from the seeds and cultured in modified MS_1 and MS_2 media and in 4 different auxin types (Dicamba, 2.4-D, 2.4.5-T, Picloram). In this experiment conducted to select the best auxin type, callus formation percentages (%) and callus weight (mg/explant) of the genotypes were determined 4 weeks after the beginning of culture.

Callus formation percentage; average values belonging to the callus formation percentages of 4 different auxin types of MS_1 and MS_2 media were given in Table I.

For each of the three genotypes, MS_1 medium was observed to present better results in terms of callus formation percentages with the values of Karatay-94 14.2%, Bülbül-89 13.3% and KxB hybrids 14.6%.

In a study [8] cultured mature embryo of different barley types, investigated the effect of media on callus formation and determined that modified MS medium containing 0.5 g/l proline and 0.5 g/l casein hydrolysates was the optimum medium for all the genotypes. In a previous experiment [3] cultured mature embryo of barley, oats, and triticale varieties, used modified MS medium containing different growth regulators (MS salts + B5 vitamins + 3% maltose + 1g/l casein hydrolysates + 0.7 g/l proline + 5 µM copper sulfate) and obtained healthy calli.

In terms of callus formation, the best result among the growth regulators were obtained in the medium containing dicamba with the results 20% for Karatay-94 type, 18.3% for Bülbül-89, and 24.2% for KxB hybrid.

In a previous experiment [9] about a regeneration study conducted by using immature embryo of barley varieties, it was stated that dicamba provided better results compared with others. Halamkova et al. [10] determined that dicamba was more effective on callus formation in immature embryo of barley and plant regeneration in comparison to 2.4-D. When we look at the results of the experiment, it was seen that dicamba provided better results in the genotypes we used and these results are in accordance with the literature. Therefore, in our study, dicamba growth regulator was used. However; some researchers stated that 2.4-D is more effective than dicamba. In a study [11] stated that in most of the barley types, media containing 2.4-D, in comparison to dicamba and picloram, has a better effect on callus formation and plant regeneration and the best results were obtained in the media containing 2,4-D. Ozgen et al. [12] succeeded in embryo culture study conducted with different 15 barley genotypes with using media contained 2,4-D.

Callus weight; average values of callus weight (mg/explant) occurred in 4 different auxin types of MS_1 and MS_2 media were given in Table II.

While MS_1 medium provides the best result with the values 14.5 mg/explant for Karatay-94 and 11.3 mg/explant for Bülbül-89, for KxB hybrid, MS_2 provided the best result with the value of 18.7 mg/explant. Based on the results herein, in the research conducted by [1], modified MS (MS_2 medium) was used and therefore, in all the working genotypes, at the rate of 25% and 55% callus was obtained and they stated that this medium shortens the time for plant regeneration. Therefore, in our study, media coded as MS_1 and MS_2 formed by modifying the MS basic medium were used as media.

When we look at the callus weight of the growth regulators, the best results for all genotypes were observed in the media containing dicamba with the values of 12.9 mg/callus for Karatay-94, 14.1 mg/explant for Bülbül-89, 30.8 mg/explant for KxB hybrid. When we look at the interaction of the media x growth regulator, the best callus weight was obtained from MS_1 + Dicamba (17.5 mg/explant) for Karatay-94; MS_1 + Dicamba (18.3 mg/explant) for Bülbül-89, and MS_2 + Dicamba (45.0 mg/explant) for KxB hybrid.

In an experiment by Ozgen et al. [12], the effects of hybrid on callus weight through mature embryo culture of different barley genotypes of parent and F_1 hybrids were studied and it was determined that there was a positive effect of hybrid strength on all characteristics except callus weight. In our study, the highest callus weight was obtained from KxB hybrid genotype (45.0 mg/explant). The positive effect of the hybrid strength was observed. It was thought that this situation may have resulted from the effect of the genotype difference.

By taking into consideration the healthy appearance of the calli and the fact that the best result for all genotypes was dicamba, different concentrations of dicamba were used in the later stages of the experiment. As in various studies [1], [11], it was stated that 2,4-D provided better results than dicamba, 2,4-D was also preferred as another auxin source in our study.

Also, the effects of all the auxins on the embryo were observed throughout the experiment. Picloram and 2.4.5-T auxins could not prevent germination which is instinctual in the embryo; on the contrary, long shoots and short pennate rooting were occurred as well as low rate of callus formation in both auxins. In the media containing dicamba and 2,4-D auxins, this situation was not observed; germination ability of the embryo was restricted.

TABLE I
CALLUS FORMATION OF DIFFERENT BARLEY GENOTYPES PERCENTAGES (%)

Genotypes	Karatay-94			Bülbül-89			KxB Hybrid		
PGR (2 mg/l)	MS_1	MS_2	Ort.	MS_1	MS_2	Ort.	MS_1	MS_2	Ort.
Dicamba	23.3	16.7	20.0a	20.0	16.7	18.3a	23.3a	25.0a	24.2a
Picloram	15.0	11.7	13.3b	11.7	10.0	10.8b	15.0b	10.0b	12.5b
2,4,5-T	11.7	11.6	11.7b	10.0	1.7	5.8c	6.7cd	0.0c	3.3c
2,4-D	6.7	1.6	4.2c	11.7	13.3	12.5b	13.3b	3.3bc	8.3bc
Average	14.2a	10.4b		13.3a	10.4b		14.6a	9.6b	
	$LSD_{\%1}$PGR: 4.553			$LSD_{\%1}$PGR: 4.553			$LSD_{\%1}$PGR: 5.442 $LSD_{\%5}$Mx PGR: 5.586		

TABLE II
CALLUS WEIGHT OF DIFFERENT BARLEY GENOTYPES (MG/EXPLANT)

Genotypes	Karatay-94			Bülbül-89			KxB Hybrid		
PGR (2 mg/l)	MS_1	MS_2	Ort.	MS_1	MS_2	Ort.	MS_1	MS_2	Ort.
Dicamba	17.5 a	8.4 b	12.9 a	18.3 a	10.0 bc	14.1 a	16.7 b	45.0 a	30.8 a
Picloram	10.7 b	7.3 b	9.0 bc	8.4 bc	8.3 bc	8.3 b	8.3 bc	16.7 b	12.5 b
2,4,5-T	15.1 a	7.4 b	11.3 ab	7.3 c	0.0 d	3.6 c	5.0 bc	0.0 c	2.5 b
2,4-D	14.7 a	0.0 c	7.3 c	11.1 b	9.0 bc	10.0 b	10.0 bc	13.3 bc	11.7 b
Average	14.5 a	5.8 b		11.3 a	6.8 b		10.0 b	18.7 a	
	$LSD_{\%1}$PGR: 2.781 $LSD_{\%1}$Mx PGR: 3.932			$LSD_{\%1}$PGR: 2.579 $LSD_{\%1}$M.x PGR: 3.647			$LSD_{\%1}$PGR: 10.04 $LSD_{\%1}$M.x PGR: 14.19		

B. Determination the Callus Induction Media

After determining that dicamba and 2,4-D were suitable auxins, with the purpose of determining the most suitable concentration and medium for callus induction, mature embryos were cultured in MS_1 and MS_2 media containing 3 different concentrations of both auxins (2, 4, 6 mg/l) 4 weeks after the initial culture, callus formation percentages (%) and callus weights (mg/explant) of each of the three genotypes were determined.

Callus formation percentage; average values belonging to the callus formation percentages occurred in 3 different concentrations of 2 different auxin types in MS_1 and MS_2 media were given in Table III.

It was determined that MS_1 media provided a better result in comparison to MS_2 media with the values 12.0% for Karatay-94 and 19.4% for KxB hybrid. The different between MS_1 and MS_2 media for Bülbül-89 type was statistically insignificant.

In the species belonging to the *Gramineae* family, it is known that the content of the media is one of the important factors that affect the callus induction and plant regeneration [9]. When reviewing the studies about the embryo culture, many researchers modified [8], [13] the nutrient element content of the media they used and tried to optimize the most suitable media for the genotype they work on. Proline, glutamine, casein hydrolysates and copper sulphate chemicals added to the media in barley embryo culture were determined to increase the regeneration capacity [14], [9], [1], [8]. Media used in our study were modified based on these studies and obtained positive results from them. In a study [13] cultured the embryo of different wheat genotypes in three different media, stated that media was effective in callus formation. In our study, the result that the media which provides the best result differs is in accordance with the results of the previous studies done by the various researchers [8], [10], [15].

When evaluating the effect of growth regulators on callus formation percentage, dicamba had the best results in Karatay-94 and Bülbül-89 varieties as 11.4% and 15.3%, respectively, while 2,4-D had the best result in KxB hybrid as 20.7%. Halamkova et al. [10] cultured the embryo of different barley genotypes in the media containing different auxins and reported that dicamba was more suitable in terms of callus formation and regeneration in comparison to 2.4-D. In contrast, [11] investigated the effect of three different auxins (dicamba, picloram, 2.4-D) on callus promotion and later regeneration capacity in different barley types, reported that they obtained the highest average of callus formation in the media containing 2.4-D in comparison to the media containing dicamba and picloram. In a study with immature embryos of three inbred rye lines [16], it was stated that there were different effects of growth regulators on callus formation and media contained 2,4-D is more important than media contained dicamba and picloram.

When we look at the interaction of media x growth regulator, the highest callus formation percentages for Karatay-94; MS_1+ dicamba (16.3%), for Bülbül-89; MS_1+ dicamba (16.2%), and for KxB hybrid; MS_1+ dicamba (19.6a) were in the same group statistically and were obtained from MS_2+ 2.4-D (22.2a%).

In a study [17] cultured mature barley embryo with different growth regulators in 4 media whose nutrient element

contents are different and according to the results of the research, they stated that while the highest callus formation was obtained from J25-8 medium containing 2 mg/l 2.4-D with the value 75.5%, this value decreased to 35% in MS medium containing 2.4-D in the same concentration; therefore, the media and growth regulators were effective together. Our research also shows that the interaction of media x growth regulators effective. Therefore, it can be stated that effects of the medium and growth regulators can change for each genotype and this needs to be taken into consideration when developing protocols.

When interpreting the interaction of growth regulator x concentration, it was determined that the media containing 4 mg/l dicamba for Karatay-94 type, 4 mg/l 2.4-D for KxB hybrid provided better results.

In [15] studying on wheat, barley, and triticale investigated the effect of 3 mg/l concentrations and combinations (1 mg/l picloram + 1 mg/l 2,4-D; 1.5 mg/l picloram + 1.5 mg/l dicamba; 1.5 mg/l picloram +1.5 mg/l 2,4-D; 1.5 mg/l dicamba+1.5 mg/l 2,4-D) of the three different auxin types (2,4-D, dicamba, picloram) on callus formation and plant regeneration. As a result of the research, they determined that the suitable auxin type and concentration depend on the genotype. In an experiment conducted in embryo culture study of different 15 barley genotypes [12], media contained 3 mg/l 2.4-D had the best results. In a study [18] investigated the effect of different 2,4-D concentrations for barley both on callus formation and plant regeneration and reported that 10 mg/l 2,4-D showed a positive effect. In a previous experiment [19] investigated the effect of different concentrations of 2.4-D and dicamba for different barley genotypes on callus formation and stated that 4 mg/l dicamba provided the best result. In a study [20] cultured mature embryo of different wheat genotypes in the media containing different dicamba concentrations and stated that 4 mg/l dicamba provided the best result. They recognized that the results were highly affected by the genotype and dicamba concentration. As can be understood from the studies, suitable auxin type and concentration depend on the genotype, and our findings also confirm these results.

Finally, when we look at the interaction of media x growth regulator, the highest callus formation percentages were obtained for Karatay-94 from MS_1 2 mg/l dicamba (18.9%), for Bülbül-89 from MS_1 + 2 mg/l dicamba (18.8%), and for KxB hybrid from MS_2 4 mg/l + 2,4-D (30.0%).

Sharma et al. [1] cultured mature embryo of different barley varieties in modified MS medium (MS_2), experimented with the different combinations of 2.4-D for callus formation, and when we look at the callus formation percentages, they obtained the best result from the medium containing 6 mg/l 2.4-D. Our study shows that the most suitable medium, growth regulator, and concentration depend on the genotype. This situation can be explained with the fact that genotype difference is the determining characteristic and depending on the genotype, this interaction can change. In a study [21] also stated that the regeneration protocols shall be developed and used for each genotype.

Callus weight; average values of callus weight occurred in 3 different concentrations of 2 different auxin types in MS_1 and MS_2 media were given in Table IV.

MS_1 media provided the best results with the values 15.6 mg/explant for Karatay-94 type, 15.8 mg/explant for Bülbül-89 type, and 24.1 mg/explant for KxB hybrid.

In a study [20] cultured mature embryo of different wheat genotypes in different nutrient media and reported that nutrient media highly affected the callus weight.

When the growth regulators were compared to the each of the genotypes, while dicamba provided the best results for Karatay-94 type (15.6 mg/explant) and Bülbül-89 type (22.1 mg/explant), 2.4-D provided the best result for KxB hybrid (23.1 mg/explant).

In a previous experiment [22] investigated the effect of different concentrations of 4 different auxin types; 2,4-D, dicamba, picloram, and 2-MCPP [2-(2-methyl-4-chlorophenol) propionic acid] for mature embryo of wheat on callus weight. They determined that the effect of auxin type on callus weight is important. Increase in picloram and dicamba concentration increased the weight of the callus, in spite of this, increase in 2,4-D concentration decreased the callus weight. Contrary to this, [23] cultured mature embryo of different corn genotypes in the media containing different concentrations of 2.4-D, reported that callus weight changed depending on the genotype and 2 mg/l and 4 mg/l concentrations provided the best results.

When we look at the interaction of the medium x growth regulator, the highest callus weight was obtained from MS_1 + Dicamba (24.6 mg/explant) for Karatay-94; MS_1 + Dicamba (28.0 mg/explant) for Bülbül-89, and MS_1 + Dicamba (25.5 mg/explant) for KxB hybrid. When growth regulator x concentration interaction was interpreted, 6 mg/l dicamba (22.8 mg/explant) for Karatay-94 type, 6 mg/l dicamba (26.5 mg/explant) for Bülbül-89 type, and 4 mg/l 2.4-D (23.3 mg/explant) concentrations were determined to provide the best results.

Finally, when we look at the interaction of medium x growth regulator, the highest callus weight was obtained from MS_1 + 6 mg/l dicamba medium for all genotypes with the values 39.1 mg/explant for Karatay-94 type, 46.3 mg/explant for Bülbül-89 type, and 30.0 mg/explant for KxB hybrid. When these results were evaluated, the growth regulator that affected our genotypes positively was dicamba, MS_1 medium was the medium.

C. Determination the Shoot Induction Media

Calli obtained in callus promotion media were cultured in MS_1medium containing one of the TDZ or BAP cytokines in order to be able to promote shoot formation. As MS_1 medium provided the best results generally in our study, we continued only with MS_1 medium at this stage of our study. 4 weeks after the beginning of culturing, the percentages of shoot growth were calculated.

When Table V was examined, it was observed that the growth regulator in the medium where the calli was taken was highly effective in shoot formation. In calli taken from the

media containing 4 mg/l 2,4-D and 4 mg/l dicamba, shoot formation occurred at a higher rate. When we compared these two growth regulators, in all genotypes for calli taken from the media containing dicamba, shoot formation occurred at a higher rate. In a study[24] cultured mature embryo of different wheat varieties of Iraq origin, they determined that there was a relationship between the callus weight and the number of regenerative plant formed out of callus and reported that with the increase of callus weight the ability to regenerate also increases. In our study, when the highest callus weights are evaluated in a general sense, they were obtained from the media containing dicamba growth regulator, that is, our result confirms the information.

TABLE III
CALLUS FORMATION OF DIFFERENT BARLEY GENOTYPES PERCENTAGES (%)

		Karatay-94			Bülbül-89			KxB Hybrid		
PGR	**Concentration**	**MS_1**	**MS_2**	**Ort.**	**MS_1**	**MS_2**	**Ort.**	**MS_1**	**MS_2**	**Ort.**
Dicamba	**2**	18.9a	4.3gh	11.5a	18.8a	17.7a	18.3	17.7b	17.7b	17.7bc
	4	16.6ab	8.8ef	12.7a	14.4ab	14.4ab	14.4	21.1b	10.0c	15.5bc
	6	13.3bcd	6.6fg	9.9a	15.5ab	11.1bc	13.3	20.0b	7.7c	13.8c
Average		16.3a	6.6b	**11.4a**	16.2a	14.4a	**15.3a**	19.6a	19.2a	**15.7b**
2,4-D	**2**	0.0h	0.0h	0.0b	4.4d	14.4ab	9.4	20.0b	18.8b	19.4b
	4	14.4bc	10.0def	12.2a	10.0bcd	7.7cd	8.9	20.0b	30.0a	25.0a
	6	8.8ef	11.1cde	10.0a	4.4d	15.5ab	10.0	17.7b	17.7b	17.7bc
Average		7.7 b	7.0b	**7.4b**	6.3b	12.5a	**9.4b**	11.8b	22.2a	**20.7a**
		$LSD_{\%1}$M.x PGR:2.528 $LSD_{\%1}$PGRxC:3.096 $LSD_{\%1}$M.xPGRxC:4.378			$LSD_{\%1}$M.x PGR:4.506 $LSD_{\%5}$M.xPGRxC:5.759			$LSD_{\%1}$M.x PGR:3.683 $LSD_{\%1}$PGRxC:4.511 $LSD_{\%1}$M.xPGRxC:6.379		

TABLE IV
CALLUS WEIGHT OF DIFFERENT BARLEY GENOTYPES (MG/EXPLANT)

		Karatay-94			Bülbül-89			KxB Hybrid		
PGR	**Concentration**	**MS_1**	**MS_2**	**Ort.**	**MS_1**	**MS_2**	**Ort.**	**MS_1**	**MS_2**	**Average**
Dicamba	**2**	19.5b	7.2cd	13.3b	28.9b	24.7b	26.8a	24.4bc	15.5e	20.0
	4	15.3bc	8.0cd	11.6b	8.4cd	17.7bc	13.1b	22.2bcd	18.9de	20.5
	6	39.1a	6.6cd	22.8a	46.3a	6.6d	26.5a	30.0a	6.6f	18.3
Average		24.6a	7.3b	**15.6a**	28.0a	16.4b	**22.1a**	25.5a	13.7b	**19.6b**
2,4-D	**2**	0.0d	0.0d	0.0c	1.1d	6.2d	3.7c	23.3bcd	22.2bcd	22.7
	4	14.1bc	7.7cd	10.9b	8.1cd	4.4d	6.2bc	20.0cde	26.6ab	23.3
	6	6.0cd	19.6b	12.8b	1.6d	2.5d	2.1c	24.4bc	22.2bcd	23.3
Average		6.6b	9.1b	**8.2b**	3.6c	4.4c	**4.0b**	22.6a	23.7a	**23.1a**
		$LSD_{\%1}$M.x PGR:6.650 $LSD_{\%5}$PGRxC:6.010 $LSD_{\%1}$M.xPGRxC:11.52			$LSD_{\%1}$M.x PGR:6.144 $LSD_{\%1}$PGRxC:7.524 $LSD_{\%1}$M.xPGRxC:10.64			$LSD_{\%1}$M.x PGR:3.858 $LSD_{\%5}$M.xPGRxC:4.931		

There are great differences between the percentages of the shoot formation and genotypes. While the highest shoot formation rate among the genotypes occurred in KxB (13.3%) hybrid, the lowest shoot formation rate was observed in Bülbül-89 (1.8%) type. This situation was thought to may have resulted from the differences in regeneration capacities of the genotypes.

When the research done on the embryo culture is examined, in order to be able to provide shoot promotion from callus, different researchers preferred different growth regulators and combinations. In a study [1] cultured the mature embryo of different barley genotypes, they investigated different combinations of BAP and TDZ growth regulators for shoot formation from calli and determined that lower rate of BAP was highly effective. Shan et al. [25] investigated in vitro regeneration in immature embryo of barley, they determined that 1 mg/l TDZ increased degeneration and supported shoot regeneration from callus. In a previous experiment [3] cultured mature embryo of different barley varieties for shoot formation in the media containing TDZ and/or BAP growth regulators and the best results were obtained from the medium containing 1 mg/l TDZ + 1 mg/l BAP for two-row barley genotype and from the medium containing 1 mg/l TDZ + 2 mg/l BAP for six-row barley genotype. On the contrary, in a study in order to determine the effects of growth regulators (BAP and TDZ) on shoot formation of mature embryo culture [26], there was no statistically significance of regeneration media contained TDZ and BAP in 0.1 mg/l or 1 mg/l concentrations on shoot formation. These results show the importance of the genotype as well as growth regulator and concentration in regeneration capacity.

IV. CONCLUSION

Based on the findings obtained from the study, it is found suitable to use 4 mg/l dicamba as the auxin type and concentration for callus formation and 1 mg/l BAP as the cytokine type and concentration for shoot formation in the nutrient medium MS_1 although it changes depending on the genotype. In this study in which embryo culture was formed from mature embryo, important findings were obtained in

terms of callus formation, callus weight, and shoot formation. However, it is at a lower rate when compared to the studies in which embryo the regeneration capacities of which are immature were used. Therefore, it is thought to be beneficial to conduct research to improve the regeneration capacity.

TABLE V
SHOOT FORMATION OF DIFFERENT BARLEY GENOTYPES PERCENTAGES (%)

Callus formation media		Shoot formation media					
PGR	**Concentration (mg/l)**	**Control**	**0.2 mg/l TDZ**	**0.5 mg/l TDZ**	**0.5 mg/l BAP**	**1 mg/l BAP**	**Average**
				Karatay-94			
	2	0.0d	0.0d	0.0d	0.0d	0.0d	0.0c
Dicamba	4	0.0d	13.3bc	13.3bc	20.0b	86.6a	26.7a
	6	0.0d	0.0d	0.0d	0.0d	13.3bc	2.7c
Average		0.0c	4.4bc	4.4bc	6.6b	33.3a	**9.8a**
	2	0.0d	0.0d	0.0d	0.0d	0.0d	0.0c
2,4-D	4	0.0d	13.3bc	0.0d	20.0b	6.6cd	8.0b
	6	0.0d	0.0d	0.0d	0.0d	0.0d	0.0c
Average		0.0c	4.4bc	0.0c	6.6b	2.2bc	**2.7b**
				$LSD_{\%1}$PGRxC:5.016 $LSD_{\%1}$PGRxS.N.:6.476 $LSD_{\%1}$PGRxCxS.N.:11.22			
				Bülbül-89			
	2	0.0d	0.0d	0.0d	0.0d	0.0d	0.0
Dicamba	4	0.0d	0.0d	0.0d	13.3bc	26.6a	8.0
	6	0.0d	0.0d	0.0d	0.0d	13.3bc	2.6
Average		0.0c	0.0c	0.0c	4.4bc	13.3a	**3.5a**
	2	0.0d	0.0d	0.0d	0.0d	0.0d	0.0
2,4-D	4	0.0d	0.0d	0.0d	6.6cd	20.0ab	5.3
	6	0.0d	0.0d	0.0d	0.0d	0.0d	0.0
Average		0.0c	0.0c	0.0c	2.2bc	6.6b	**1.8b**
				$LSD_{\%1}$PGRxS.N.:5.287 $LSD_{\%1}$PGRxCxS.N.:9.158			
				KxB Hybrid			
	2	0.0e	0.0e	0.0e	0.0e	0.0e	0.0d
Dicamba	4	0.0e	20.0bc	20.0bc	26.6b	100.0a	33.3a
	6	0.0e	0.0e	0.0e	13.3cd	20.0bc	6.6c
Average		0.0d	6.6c	6.6c	13.3b	40.0a	**13.3a**
	2	0.0e	0.0e	0.0e	0.0e	0.0e	0.0d
2,4-D	4	0.0e	6.6de	6.6de	20.0bc	26.6b	12.0b
	6	0.0e	0.0e	0.0e	0.0e	26.6b	5.3c
Average		0.0d	2.2cd	2.2cd	6.6c	17.7b	**5.7b**
				$LSD_{\%1}$PGRxC:5.016 $LSD_{\%1}$PGRxS.N.:6.476 $LSD_{\%1}$PGRxCxS.N.:11.22			

ACKNOWLEDGMENT

This study was supported by Selcuk University Scientific Research Projects (Selcuk University-BAP, Konya-Turkey, Project Number: 13101015). The authors wish to thank BAP staffs.

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