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Investigation of the effects of different genotypes on regeneration capacity in Triticale

Ismail Bezirganoglu[1*], Serap Karaman[1], Beyza Reisoğlu[1], Fatma Böke[1], Büşra Yazıcılar[1]

[1]*Department of Molecular Biology and Genetics, Erzurum Technical University, 25050 Erzurum, Turkey.*

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Abstract

Triticale (x Triticosecale Wittmack) is a synthetic amphiploid cereal that grows on about 3 million hectares in the world. It is grown mostly for forage or animal feed, although some triticale-based foods can be purchased at health food stores or are found in some breakfast cereals. Mature embryos of two triticale cultivars (Ümran Hanım and Melez 2001) were used as the resources explants. The effects of one auxin type (2,4-D) and three various concentrations, (4.0 mg/l; 8mg/l; 12.0mg/l) callus formation and plant regeneration were determined. Callus formation values were detected over 85% in three concentrations. In terms of results, the highest embryogenic callus formation rate was determined 46.91 % at 12 mg/l of Ümran Hanım dicamba. However, the lowest embryogenic callus formation rate was found with a value of 12.86% in Ümran Hanım's 2,4-D 8.0 mg/l hormone application. The highest regeneration capacity was determined at 12.09% at a dose of 12.0 mg/l of Mikham 2001 2,4-D. However, the lowest regeneration capacity was determined at 2.2% at the dose of 4 mg/l of Ümran Hanım 2,4-D. Our results displayed that auxin type and hormone dosage were very significant on the triticale mature embryos.

Keywords: In vitro culture, Plant regeneration, Callus, Hormones.

Introduction

Increasing plant production is an inevitable reality in order to meet the nutritional needs of the constantly increasing human population. Although many varieties with yield capacity and quality have been developed and brought to the food sector by utilizing traditional plant breeding studies, the desired result has not been fully achieved in resistance to some biotic and abiotic environmental stress, especially diseases and pests. With the advancement of technologies in the field of genetic engineering and the understanding of molecular plant protection mechanisms, new technologies have been developed in the control of stress factors. Cell and tissue culture techniques are a very effective method that uses molecular and cellular-based special technologies to increase the productivity of plant and plant products, or to eliminate the factors

* *Corresponding Author:* Ismail Bezirganoglu,
Department of Molecular Biology and Genetics,
Erzurum Technical University, 25050 Erzurum, Turkey.
E-mail: ismail.bezirganoglu@erzurum.edu.tr

that cause abiotic and biotic stresses, thus preventing plant yield losses [1-4]. In triticale breeding studies, there are intensive studies on agronomic characters such as grain yield, plant height and nutrient content obtained by classical methods, and a good regeneration method should be established from tissue and cell cultures in order to benefit effectively from biotechnological applications used in plant breeding [5]. Therefore, the success of tissue and cell research depends on a sustainable and effective callus culture and plant regeneration process. Immature embryos are generally used as an explant source for somatic callus culture in cereals. Progress has been made in changing the agronomic characteristics of cultivated plants and developing varieties resistant to stress factors, with tissue culture methods that eliminate adverse environmental conditions under controlled conditions developed in recent years [6-8]. Although the success rate in tissue culture is lower in monocotyledons, especially in the Graminea family, than dicotyledons, today it is used in bread wheat [9], maize [10], rice [11] and barley [12] plant regeneration was achieved. There are relatively few studies on the in vitro regeneration of triticale using mature and immature embryos compared to other cereal species. The lack of an effective in-vitro system limits tissue culture studies. The factors that determine the response to tissue culture in cereals, including triticale, are the components of the donor plant, the development status of the explant, and the culture medium [13]. Although there is a successful protocol for plant regeneration using embryos of mature seeds with triticale, there are very few studies on callus formation and regeneration frequency using different plant growth regulators. In many reports on callus regeneration, the most commonly used plant growth regulator to stimulate somatic embryogenesis is 2,4-D (2,4-Dichlorophenoxy acetic acid), picloram (4-amino-3,5,6-trichloropicolinic acid) and dicamba (3,6-Dichlorobenzoic acid) are also used alone or together with 2,4-D [14]. In a study of triticale immature embryo culture, picloram was superior to 2,4-D in embryogenic callus formation and plant regeneration [15]. In another study, it was noted that dicamba provided callus formation faster than 2.4-D and plant regeneration was 2 times higher from the formed calli [16]. In a study of immature embryo culture in wheat, dicamba was found to be better than 2,4-D and picloram in terms of embryogenic callus formation and plant regeneration [17]. Ma and Pulli, 2004 indicated that dicamba was found to be more effective in embryogenesis than 2,4-D and NAA (Naphthalene acetic acid) in immature embryo culture in the rye [18]. The aim of this study is to improve callus formation and plant regeneration efficiency by developing an effective callus formation and the plant regeneration system from mature embryos in triticale and to determine the response of some triticale genotypes to tissue culture of different plant growth regulators and hormone concentrations.

Materials and Methods

Plant material and culture condition: *Ümran Hanım and Melez 2001* genotypes were provided from East Anatolia Agricultural Research Institute. In this report, mature embryos of Ümran Hanım and Mikham 2001 genotypes were used as explants. The mature seeds were mixed in 70% ethanol for 5 minutes and washed 3 times with sterile distilled water. It was then mixed in 1% sodium hypochlorite containing a few drops of Tween 20 (Sigma) for 20 minutes. After surface sterilized seeds were washed with sterile distilled water, they were kept in sterile distilled water at 25°C for 3 hours in the dark. Mature embryos were obtained for callus formation include MS (Murashige and Skoog, ascorbic acid) containing 2 different auxin types (2,4-D, and dicamba), 3 different doses (4.0, 8.0 and 12.0 mg/l), agar (8 g/l) and 1.95 g/l MES. The pH of the medium was adjusted to 5.8 with NaOH (Sodium hydroxide). A factorial experiment was conducted in a

completely randomized design with three replications. Twenty five mature embryos were placed in each petri dish and each petri dish was accepted as an experimental unit. Callus formation (%) [CF% = (Number of callus formed/Explant number) x100], embryogenic callus formation (%) (ECF% = (Embryogenic callus number/ Callus number) x100] was determined.

Results

Callus formation: The callus formation rate ranged from 83.69-93.77% in all application hormone concentrations of two different genotypes used in the experiment (data not shown).

Embryogenic Callus Formation: Calli was evaluated as embryogenic and non-embryogenic calli, whereas embryogenic ones had the potential to form somatic embryos. Callus formation was observed in over 80% of all explants cultured. The difference

Table 1. Embryogenic callus formation ration in terms of callus formation (%)

Hormones	Dose (mg/l)	Genotype		Mean
		Mikham 2001	Ümran Hanım	
2,4-D	4,0	19.3+6.12	14.08+8.06	16.65
	8,0	14.17+6.46	12.86+5.17	13.51
	12,0	33.98+8.02	32.38+9.17	33.18
	Mean	22.48+10.47	19.77+11.11	21.12
Dicamba	4,0	25.04+6.48	39.47+3.92	32.25
	8,0	17.80+2.89	39.45+6.32	28.62
	12,0	35.49+9.33	46.91+7.91	41.2
	Mean	26.11+9.78	41.95+6.85	34.03
Mean		24.30+10.29	30.86+14.47	27.58

between genotypes in terms of embryogenic callus formation rate, according to callus number was found to be statistically significant ($p<0.05$). When the general averages of auxin types and doses are evaluated according to genotypes, it is seen that the rate of callus formation rate is Ümran Hanım 30,863 and Mikham 2001 24,300% (Table 1). While the embryogenic callus formation rate was 19.77% in the 2,4-D application, it was 41.95% in the dicamba application. In Mikham 2001, these rates were 22.48% and 26.11% in media containing 2,4-D and dicamba, respectively. Considering the combination of all the factors used in the experiment, the highest embryogenic callus formation rate (46.91%) was determined at 12 mg/l of Ümran Hanım dicamba. On the other hand, the lowest embryogenic callus formation rate was found with a value of 12.86% in Ümran Hanım's 2,4-D 8.0 mg/l dose (Table 1).

Table 2. Embryogenic callus formation ratio in terms of auxin (%)

Genotypes	Mean (mg/l)			
	4.0 mg/l	8.0 mg/l	12.0 mg/l	Mean
Mikham 2001	22,17	15,98	34,73	24.29
Ümran Hanım	26,77	26,16	39,65	30.86
Mean	24.47	21.07	37.19	27.575

The embryogenic callus formation ratio in terms of 4.0 mg/l, 8.0 mg/l and 12.0 mg/l dose applications, Ümran Hanım was obtained 26.77%, 26.16% and 39.65%, whereas Mikham 2001 was obtained as 22.17%, 15.98% and 34.73%, in the same order (Table 2).

Regeneration Capacity: Embryogenic calli with green plantlets were evaluated as embryogenic calli with regeneration capacity. When the general averages of auxin types and doses were evaluated according to genotypes, the rate of regeneration capacity in terms of the number of callus was 6.33% for Ümran Hanım and 7.15% for Mikham 2001. The rate of regeneration capacity, according to the number of callus in Ümran Hanım was 4.91% in the 2,4-D application and 7.74% in the dicamba application. In Mikham 2001, these rates were 5.54% and 8.76%, respectively, Considering the combination of all the factors used in the experiment, the highest regeneration capacity (12.09%) was determined at a dose of 12.0 mg/l of Mikham 2001 2,4-D. On the other hand, the lowest regeneration capacity (2.2%) was determined at the dose of 4 mg/l of Ümran Hanım 2,4-D (Table 3). Regeneration capacity in terms of 4.0 mg/l, 8.0 mg/l

and 12.0 mg/l dose applications, Ümran Hanım was obtained 3.5%, 6.3% and 9.03%, whereas Mikham 2001 was obtained as 6.7%, 5.3% and 9.3%, in the same order (Table 4).

Table 3. Plant regeneration ratio in terms of callus formation (%)

Hormones	Dose (mg/l)	Genotype		Mean
		Mikham 2001	Ümran Hanım	
2,4-D	4,0	4.72+3.11	2.25+3.32	3.48
	8,0	5.30+3.86	3.72+3.90	4.51
	12,0	6.60+3.87	8.76+6.28	7.68
	Mean	5.54+3.46	4.91+5.21	5.22
Dicamba	4,0	8.71+3.67	4.87+4.46	6.79
	8,0	5.48+3.76	9.05+7.91	7.26
	12,0	12.09+3.49	9.30+6.21	10.69
	Mean	8.76+4.38	7.74+5.06	8.25
Mean		7.15+0.78	6.33+5.24	6.74

Table 4. Plant regeneration ratio in terms of auxin doses (%)

Genotypes	Dose (mg/l)			
	4.0 mg/l	8.0 mg/l	12.0 mg/l	Mean
Mikham 2001	6,72	5,30	9,34	7.15
Ümran Hanım	3,56	6,39	9,03	6.33
Mean	5.14	5.89	9.19	6.74

Discussion

Success in callus formation and plant regeneration studies in many plant species, including cereals largely depends on genotype, explant type and media components. Genotype is the most important factor affecting the somatic embryogenesis of cereals from mature (19, 20, 5). In this study, it was determined that there were significant differences between the genotypes used on embryogenic callus formation and the number of regenerated plantlets. In similar studies on this subject, it has been reported that the effect of genotype on embryogenic callus formation is very important, according to callus number [21, 22]. These findings are in agreement with those published by Rakoczy-Trajanonowska and Malepszy [23] on rye in the study of the rate of embryogenic callus formation varies according to genotype. Several reporters observed that 2 weeks after callus formation in inbred lines of rye, yellow, hard and nodular calli were formed on 2 lines, and white and watery embryogenic calli were formed in the remaining 5 lines. In a study by Aydın et al. [14] in which they investigated the factors affecting tissue culture using embryos matured in wheat, they determined that the effect of genotype on the number of somatic embryos was important and that dicamba was more effective than 2.4-D and picloram as an auxin type. The concentration of plant growth regulators in the culture medium has an important effect on morphogenesis and growth. Generally, high concentration of auxins and low cytokinin increase callus formation and cell proliferation. Regardless of the explant source, 2,4-D triticale is the most widely used plant growth regulator for callus formation and maintenance. The results of this study determined that the frequency of callus formation and embryogenic callus formation was much higher in the medium containing dicamba. Unlike the findings, we obtained in this study, Vikrant and Rashid [22] stated that low concentrations of plant growth regulators are more effective in forming somatic embryos. Zapata et al., [24] stated that non-high doses gave better results in plant regeneration from embryogenic calluses of barley. Venkant et al., [25] observed that embryogenic calli regenerated by forming somatic embryos in a dose of 2.0 mg/l and within 3-4 weeks. Considering the combination of all the factors used in the experiment, when a comparison is made between auxin types, it is seen that dicamba is in the first place in terms of regeneration capacity. Papenfus and Carman [26] determined that dicamba was more effective than 2,4-D on regeneration capacity in immature embryo culture in wheat. Satyavathi et al., [17] reported that dicamba was more effective than 2,4-D and picloram in plant regeneration as well as in callus formation. Similarly, Mendoza and Kappler [27] found that the number of plants per embryo was 2 times higher in the

medium containing dicamba in mature embryo culture in wheat. Zimny and Lörz [13] tested immature embryos in rye with different doses of different auxins for MS [28], N6, CC, and B5 in four different basic media to determine somatic embryogenesis and plant regeneration, and CC containing 30 µM dicamba bested obtained in the environment. Similar to the results mentioned above, it was concluded that dicamba is more effective than 2,4-D in terms of embryogenic callus formation and plant regeneration, but our results show that high-dose applications rather than low doses of dicamba play an active role in creating higher regeneration. Mature embryos of Ümran Hanım and Mikham 2001 genotypes of triticale were tested in MS medium with different plant growth regulators and at different doses in order to determine callus, embryogenic callus formation and regeneration capacity. Callus formation rate, embryogenic callus formation according to callus number, regeneration capacity rates, according to callus number was investigated. According to the results obtained from this study, the effect of genotype, plant growth regulators and dose on callus formation rate was observed, and statistically significant differences were found between genotypes in terms of embryogenic callus formation rate.

Regeneration capacity, genotype, plant growth regulators and dose applications were effective, according to callus number and embryogenic callus number. According to the findings obtained from the study, it can be recommended to use high doses of 12.0 mg/l dose of Ümran Hanım genotype and dicamba, a plant growth regulator, in studies on the mature embryo culture of triticale.

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ORCID

Ismail Bezirganoglu 0000-0003-4079-5998
Serap Karaman 0000-0003-1216-0012
Beyza Reisoğlu 0000-0003-1149-9022
Fatma Böke 0000-0002-4543-6575
Büşra Yazıcılar 0000-0003-2465-7579

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