In vitro mature embryo culture protocol of einkorn (*Triticum monococcum* ssp. *monococcum*) and bread (*Triticum aestivum* L.) wheat under boron stress

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Abstract

Mature embryos of einkorn (*Triticum monococcum* ssp. *monococcum*) and bread (*Triticum aestivum* L.) wheat were used for callus induction on media containing four different doses (0, 1, 2 and 4 mg L⁻¹) of 2,4-D and dicamba supplemented with five different boron concentrations (0, 6.2, 12.4, 24.8, and 37.2 mg L⁻¹). The obtained callus was transferred to culture media with three (0, 0.5, and 2 mg L⁻¹) different BAP doses with five boron concentrations for further regeneration. The maximum callus weight in einkorn wheat was in culture media with 1 mg L⁻¹ dicamba and 6.2 mg L⁻¹ (3.71 ± 0.13 g). Bread wheat had the maximum callus weight on culture media with 4 mg L⁻¹ dicamba and 12.4 mg L⁻¹ (3.46 ± 0.40 g). The highest plantlet numbers were in only 2 mg L⁻¹ BAP (2.92 ± 0.88) for einkorn wheat and 0.5 mg L⁻¹ BAP supplemented with 6.2 mg L⁻¹ boron (3.71 ± 1.12) for bread wheat. This indirect regeneration protocol using mature embryos of einkorn and bread wheat under boron stresses expected to be useful for future wheat breeding studies.

Key message

The embryo tissues of different types of wheat have provided to develop under in vitro conditions by applying various boron concentrations. 2,4-D and dicamba, which are different auxin types, played an effective role in the callus formation.

Keywords Boron stress · Bread · Callus · Einkorn · Mature embryo

Introduction

Wheat is in the Triticeae tribe of the Poaceae family. It has a major role in all around the world not only for a nutrient source for many people but also as a feed source for animals. It is grown worldwide on 215.9 million ha, which is approximately 30% of the total agricultural land, results in a production of 765.7 million tons in 2019 (FAO, 2020).

The diploid (2n = 2x = 14, A^mA^m) cultured einkorn (*Triticum monococcum* ssp. *monococcum*) is one of the first cultivated wheat. It is derived from *T. boeoticum* in the Karacadağ mountain in Southern Eastern Turkey, about 10,000 to 13,000 years ago (Peng et al. 2011). Einkorn contains high amounts of carotenoids, antioxidants, vitamins (B and E), and minerals such as zinc, iron, and copper (Suchowilska et al. 2012; Şahin et al. 2018; Yaman et al. 2019; Pehlivan-Karakaş et al. 2020). Its protein content ranges between 16.00 and 28.50% (Hammer et al. 1996). Moreover, einkorn wheat has also useful genetic traits such as resistance to biotic/abiotic stresses (Abdel-Aal et al. 1995). These genetic traits may be advantageous in wheat breeding programs. The other important cultured wheat species are tetraploid (2n = 4x = 28, AABB) durum (*T. durum*) and hexaploid (2n = 6x = 42, AABBDD) bread (*T. aestivum* L.) wheats. These two are the most grown and consumed wheat species today.

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Global crisis such as Covid-19 pandemic showed that crop production such as wheat, rice maize etc. is highly strategic for food supply and safety. Humankind may face to a global warming crisis in near future. Global warming may, unfortunately, worsen climatic conditions, deteriorate agricultural estate conditions, decrease quality and production of the crops. To feed huge amount of human population today, new approaches should be developed to improve high yielding and biotic/abiotic stress resistant crops (Örgeç et al. 2021).

Boron is an essential micro element for plant growth and development. Plants take boron in boric acid form from the soil and groundwater. Naturally occurring boundaries, fertilization, and irrigation may cause boron availability in the soil (Nable et al. 1997). Boron toxicity is a serious abiotic stress in arid and semi-arid regions around the world (Kalayci et al. 1998) and it may be the one of the prevalent stresses in near future due to drought. In boron deficiency, properties such as growth rate, yield, and quality in plants reduce. On the other hand, the boron excess in the soil causes toxicity in plants and leads harmful effects on photosynthesis, stem cell differentiation, cell wall expansion, and deoxidative damage (Cervilla et al. 2009). Boron excess in the soil especially affect cereals such as wheat and barley (Gupta et al. 1985). As a result, the yield and quality in these grains decrease (Cartwright et al. 1986).

Genetic transformation is a good approach to improve biotic/abiotic stress resistance in wheat cultivars. Tissue culture, in that context, is a key step to a successful genetic transformation and a productive wheat breeding. Tissue culture success can be affected by many factors such as explant type, media compositions, genotype or growth conditions, etc. Any tissue culture application may show different responses even indifferent wheat cultivars (Jabeen et al. 2016). It is important to emphasize that an effective and applicable tissue culture protocol is needed for each wheat species in wheat improvement programs. Tissue culture systems for various species prefer mostly immature embryos as explants. Although immature embryo has higher callus production and regeneration, it has some restrictions such as longer production time or intensive workload (Yang et al. 2015; Mahmood and Razzaq, 2017).

Considering all these points, we focused, in this study, on determining an appropriate and effective tissue culture protocol for einkorn and bread wheat variety by testing mature embryos under five different boron concentrations and two different auxin hormones in MS basal medium. Furthermore, four different 2,4-D and Dicamba doses for callus induction, five different boron concentrations, and three doses of BAP for indirect regeneration were accomplished.

Material and methods

Seed source

Seben / Bolu 2016 harvest of mature einkorn wheat (IZA: *Triticum monococcum* sssp.. *monococcum*) and Central Research Institute for Field Crops (CRIFC) provided bread wheat (*Triticum aestivum* L.; Cv: Tosunbey) embryos were used as explant sources for callus induction and indirect regeneration.

Seed sterilization

De-hulled einkorn and bread wheat seeds were washed by five drops Tween 20 (Merck, Darmstadt, Germany supplemented) 100 mL distilled water for one minute, then, disinfected with 40% commercial bleach (4.6% NaClO; Dometos, Istanbul, Turkey) with a magnetic stirrer for 15 min in a laminar air flow. Seeds were, then, washed with sterile water to remove chemicals from seeds at the end (Örgeç et al. 2018).

The seeds were, after seed sterilization, kept in a sterile pure water bath at 33 °C for approximately 2–3 h for easy embryo extraction (Özgen et al. 1998).

Callus induction

Excised einkorn and bread wheat mature embryos were used as explants for callus induction. Callus induction media composed of 4.4 g L⁻¹ MS (Murashige and Skoog, 1962) (Duchefa-Haarlem, Netherlands), 30 g L⁻¹ sucrose (Merck Darmstadt, Germany), 8 g L⁻¹ agar (Duchefa-Haarlem, Netherlands) and five different boron (Sigma-Aldrich-USA) concentrations of 0–37.2 mg L⁻¹ (Normally MS media contain 6.2 mg L⁻¹ boron). In addition, four different doses ranging from 0 to 4 mg L⁻¹ of 2,4-D (Sigma-Aldrich, Steinheim, Germany) and Dicamba (Duchefa-Netherlands) were added to callus induction media. The pH of the medium was adjusted to 5.7 to 5.8 using 1 N HCl and 1 N NaOH before autoclaving (NC 40 M NUVE-Ankara, Turkey) at 121 °C and 1.06 kg cm⁻² pressure for 15 min. Subcultures were performed every two weeks.

Detached embryos were placed into callus induction media with scutellum upwards. Each petri dish had 10 embryos in five replicates. The culture media were kept in the growth room at 25 ± 1 °C under dark conditions for four weeks. Callus weight of calli formed after 4 weeks were measured and recorded.

Plant regeneration

Calli derived from immature seeds of einkorn and bread wheats were carried to regeneration media. The media composed of 4.4 g L⁻¹ MS, 30 g L⁻¹ sucrose, 8 g L⁻¹ agar and five different boron concentrations ranging from 0 to

37.2 mg L⁻¹ were supplemented with different BAP (6-Benzylaminopurine) (Duchefa-Netherlands) concentrations ranging from 0 to 2 mg L⁻¹ for four weeks. The culture was kept in a growth room at 25 ± 1 °C under 16-h light/8-h dark photoperiod. Subcultures were performed every two weeks. At the end of four weeks, regenerating calli in petri were counted for number of plantlets.

Acclimatization

The plantlets were washed with sterile water to remove media and then, planted in pots containing the mixture of vermiculate and peat soil at a ratio of 1:3. The plantlets were kept under the same conditions as regeneration process.

Statistical analysis

The experiment was carried out in a five-replicate randomized complete plot design with n ten embryos contained in petri dishes. Data on callus weight (g), and number of plantlets (n) were obtained. Statistical analyses were performed by using SPSS 23.0 program. The analysis of

variance (ANOVA) with Duncan post hoc test was used to determine the differences between boron doses, plant growth regulators, and species (Düzgüneş et al. 1987; Saha et al. 2017).

Results

Callus weight (g)

All mature einkorn and bread wheat embryos were reached 100% callus induction ratio except the control group (Data not shown) (Fig. 1).

Among two different auxins, dicamba responded better than 2,4-D for callus weight. One mg L⁻¹ dicamba significantly produced the highest callus weight (3.71 ± 0.13 g) for einkorn wheat cultured on MS medium supplemented with 6.2 mg L⁻¹ boron. On the other hand, for bread wheat, 4 mg L⁻¹ dicamba cultured on MS medium with 12.4 mg L⁻¹, boron responded better than all other hormone doses for the callus weight (3.46 ± 0.40 g) (Table 1).

Fig. 1 The effect of different 2,4-dichlorophenoxyacetic acid (2,4-D) and Dicamba concentrations under the different boron stresses on callus induction from mature embryos of einkorn and bread wheat. (A-I: Einkorn mature embryo, J-O: Bread mature embryo) **A** 1 mg L⁻¹ Dicamba, **B** 2 mg L⁻¹ Dicamba, **C** 4 mg L⁻¹ Dicamba (A, B and C under 0 mg L⁻¹ Boron). **D** 1 mg L⁻¹ Dicamba, **E** 2 mg L⁻¹ Dicamba, **F** 4 mg L⁻¹ Dicamba (D, E and F under 6.2 mg L⁻¹ Boron). **G** 1 mg L⁻¹ Dicamba, **H** 2 mg L⁻¹ Dicamba, **I** 4 mg L⁻¹ Dicamba (G, H and I under 12.4 mg L⁻¹ Boron). **J** 1 mg L⁻¹ 2,4-D, **K** 2 mg L⁻¹ 2,4-D, **L** 4 mg L⁻¹ 2,4-D (J, K and L under 24.6 mg L⁻¹ Boron). **M** 1 mg L⁻¹ 2,4-D, **N** 2 mg L⁻¹ 2,4-D, **O** 4 mg L⁻¹ 2,4-D (M, N and O under 37.2 mg L⁻¹ Boron)

Variance analysis showed that there was a significant interaction between wheat genotype/boron dose, wheat genotype/boron stress, and boron stress/hormone on callus weight. However, there was no significant interaction for hormone type, wheat genotype/hormone type and wheat genotype/boron stress/hormone type at 1 mg L⁻¹ hormone dose (Table 2).

As seen in Table 3, there was significant interaction between wheat genotype and boron stress and hormone type and boron stress/hormone type for callus weight. Wheat genotype/boron stress and wheat genotype/hormone type, and wheat genotype/boron stress/hormone type did not significantly interact at 2 mg L⁻¹ hormone dose.

Boron stress and hormone type and wheat genotype/boron stress and wheat genotype/hormone type had significant interactions on callus weight. Other parameters, however, did not significantly interacted at 4 mg L⁻¹ hormone dose (Table 4).

Plantlets number (n)

The highest plantlet numbers existed in 2 mg L⁻¹ BAP without boron (2.92 ± 0.88) for einkorn wheat and 0.5 mg L⁻¹ BAP supplemented with 6.2 mg L⁻¹ boron (3.71 ± 1.12) for bread wheat (Table 5).

For shoot regeneration, well-developed callus cultures derived from mature embryos of einkorn and bread wheat cultured on MS media supplemented with 5 different boron and 3 different BAP concentrations was utilized (Fig. 2). After four weeks of culturing, not only all culture media with BAP hormone but also control group regenerated (Table 5).

Boron stress significantly interacted for number of plantlets at all BAP doses. Wheat genotype/boron stress displayed a significant interaction in control group and 0.5 mg L⁻¹ BAP doses. Genotype did not, however, interacted for number of plantlets in any BAP doses (Table 6).

Acclimatization

Well-developed plants produced by indirect plant regeneration were detached from media and planted into the mixture of soil and vermiculate (3:1) for a growth in a growth room. After 49 days, bread wheat started to head and at the end of 90 days, well-developed heads were formed (Fig. 2–3). However, einkorn wheat did not head at the end of acclimatization stage.

Discussions

Optimum media composition was 1 mg L⁻¹ dicamba with 6.2 mg L⁻¹ boron for callus weight, and 2 mg L⁻¹ BAP for plantlet numbers in einkorn (*Triticum monococcum* ssp. *monococcum*). On the other hand, optimum media content

was 4 mg L⁻¹ dicamba with 12.4 mg L⁻¹ boron for callus weight, and 0.5 mg L⁻¹ BAP with 6.2 mg L⁻¹ boron for plantlet numbers in bread wheat (*Triticum aestivum* L.). Moreover, dicamba was superior than 2,4-D on callus induction for both wheat species.

Plant tissue culture was affected by genotype (Li et al. 2003), plant growth conditions (Hess and Carman, 1998), explant source (Aydın et al. 2011), and culture media (Fennell et al. 1996). Therefore, it is expected that each single plant genotype requires its own specific tissue culture protocol. The most used explant types in wheat tissue culture are immature and mature embryo (Özgen et al., 1996). In this study, we used einkorn and bread wheat mature embryos which produced 100% callus, which resulted a similarity with a previous study with callus induction in ten commercial Indian bread wheat genotypes (Parmar et al. 2012).

Agriculture is adversely affected by abiotic factors including boron all around the world. Twenty percent of agricultural lands are boron toxicity risk (Hakkı et al. 2007). Boron in lower amounts, on the other hand, is one of the essential elements for plant growth and development (Siddiqui et al. 2013). According to previous studies, the quality and yield of wheat are increased by adding the required amount of boron under conditions where the amount of boron is low (Wrobel 2009). Though, applying excessive boron can, on the other hand, decrease shoot length and weight (Marschner 1995). For all these reasons, determining the amount of boron is highly important for an efficient plant growth and development.

Plant growth regulators play an important role on embryogenic change, cell cycle regulation, and cell division. Therefore, cytokinins, auxins, with their concentrations and combinations are very important in in vitro studies (Verma et al. 2016). Dicamba, 2,4-D, and Picloram are the most used auxin plant growth regulators in wheat and other cereal mature embryo cultures for callus induction (Satyavathi et al. 2004; Filippov et al. 2006). Present study showed that dicamba was more effective on callus weight than 2,4-D. The result agreed with a previous study by Murin et al. (2012) where dicamba was more effective on callus formation at *Triticum aestivum* L. cv. 'Bobwhite'. While dicamba is more quickly taken by metabolism and improves callus development 2,4-D avoids accumulation, enzymatic degradation, and remains extremely stable in plant cells (Papenfuss and Carmen, 1987; Moore, 1989; Aydın et al. 2011).

The results here showed that callus weight, genotype and boron stress were quite significant at all 2,4-D and Dicamba doses. Species varied for callus weight. Einkorn callus was heavier than that of bread wheat. This result was supported by Nasircilar et al. (2006), where five different bread and five durum wheat callus weights differed. Both studies indicated that genotype may play an important role in callus weight. Boron dose is another important factor which

Table 1 Effects of different 2,4-D and Dicamba concentrations under boron stress on callus

Genotypes	Boron Concentrations (mg L ⁻¹)	Hormone Types	Hormone Concentrations (mg L ⁻¹)	Callus Weight (g)*			
Einkorn (<i>T. monococcum</i> sssp. <i>monococcum</i>) (IZA)	0	2, 4-D	Control (0)	—			
			1	2.57	±	0.38	ghijklmn
			2	2.20	±	0.59	mnopqrs
			4	2.59	±	0.76	ghijklmn
		Dicamba	Control (0)	—			
			1	3.10	±	0.53	abcdefg
			2	3.50	±	0.30	abcd
			4	2.70	±	0.26	fghijklm
		2, 4-D	Control (0)	—			
			1	3.54	±	0.35	abc
			2	3.03	±	0.55	cdefghi
			4	2.88	±	0.60	efghijkl
	6.2	Dicamba	Control (0)	—			
			1	3.71	±	0.13	a
			2	3.54	±	0.32	abcd
			4	3.64	±	0.16	ab
		2, 4-D	Control (0)	—			
			1	3.34	±	0.56	abcde
			2	2.59	±	1.47	ghijklmn
			4	2.23	±	0.12	mnopqrs
		Dicamba	Control (0)	—			
			1	2.92	±	0.51	defghijkl
			2	2.43	±	0.46	hijklmno
			4	2.17	±	0.26	mnopqrs
Bread Wheat (<i>T. aestivum</i> L.) (cv Tosunbey)	12.4	2, 4-D	Control (0)	—			
			1	2.57	±	0.26	ghijklmn
			2	2.32	±	0.44	klmnopqr
			4	2.29	±	0.69	lmnopqr
		Dicamba	Control (0)	—			
			1	2.02	±	0.39	nopqrst
			2	2.04	±	0.25	nopqrst
			4	2.34	±	0.37	jklmnopq
	24.6	2, 4-D	Control (0)	—			
			1	2.32	±	0.24	klmnopqr
			2	2.36	±	0.26	jklmnopq
			4	1.99	±	0.17	nopqrst
		Dicamba	Control (0)	—			
			1	2.29	±	0.29	lmnopqr
			2	2.42	±	0.47	hijklmnop
			4	2.12	±	0.55	mnopqrst
Bread Wheat (<i>T. aestivum</i> L.) (cv Tosunbey)	37.2	2,4-D	Control (0)	—			
			1	1.97	±	0.44	nopqrst
			2	2.14	±	1.02	mnopqrst
			4	1.77	±	0.35	pqrstuv

Table 1 (continued)

Genotypes	Boron Concentrations (mg L ⁻¹)	Hormone Types	Hormone Concentrations (mg L ⁻¹)	Callus Weight (g)*			
	6.2	Dicamba	Control (0)	–			
			1	2.60	±	0.40	ghijklmn
			2	3.24	±	0.37	abcdef
			4	3.04	±	0.59	bcdefgh
		2, 4-D	Control (0)	–			
			1	2.57	±	0.53	ghijklmn
			2	2.15	±	0.54	mnopqrst
			4	1.59	±	0.70	stuv
		Dicamba	Control (0)	–			
			1	2.14	±	0.19	mnopqrst
			2	2.96	±	0.27	cdefghijk
			4	3.23	±	0.39	abcdef
	12.4	2, 4-D	Control (0)	–			
			1	2.40	±	0.61	ijklmnop
			2	2.00	±	0.51	nopqrst
			4	1.53	±	0.59	tuv
		Dicamba	Control (0)	–			
			1	1.82	±	0.21	opqrstu
			2	2.58	±	0.47	ghijklmn
			4	3.46	±	0.40	abcde
	24.6	2, 4-D	Control (0)	–			
			1	2.24	±	0.69	mnopqrs
			2	1.72	±	0.34	qrstuv
			4	1.33	±	0.30	uv
		Dicamba	Control (0)	–			
			1	1.90	±	0.14	opqrstu
			2	2.43	±	0.30	hijklmno
			4	2.98	±	0.37	cdefghij
	37.2	2,4-D	Control (0)	–			
			1	2.08	±	0.27	mnopqrst
			2	1.60	±	0.41	stuv
			4	1.20	±	0.62	v
		Dicamba	Control (0)	–			
			1	1.68	±	0.17	rstuv
			2	2.23	±	0.23	mnopqrs
			4	2.61	±	0.22	ghijklmn

*Different letters indicate significant differences at $p < 0.05$

Table 2 Analysis of variance of callus weights at 1 mg L⁻¹ 2,4-D/Dicamba in mature wheat embryo

Source	Sum of Squares	Df	Mean Square	F	Sig
Genotypes	12.236	1	12.236	77.218	0.000
Boron Stress	10.466	4	2.616	16.513	0.000
Hormone Type	0.509	1	0.509	3.217	0.076
Genotypes X Boron stress	3.736	4	0.934	5.894	0.000
Genotypes X Hormone types	0.164	1	0.164	1.040	0.310
Boron stress X Hormone types	3.734	4	0.933	5891	0.000
Genotypes X Boron Stress X Hormone types	0.549	4	0.137	0.867	0.487
Error	12.676	80	0.158		
Total	42.848	99			

Table 3 Analysis of variance of callus weights at 2 mg L⁻¹ 2,4-D/Dicamba in mature wheat embryos

Source	Sum of Squares	df	Mean Square	F	Sig
Genotypes	3.964	1	3.964	19.238	0.000
Boron Stress	10.119	4	2.530	12.277	0.000
Hormone Type	5.424	1	5.424	26.324	0.000
Genotypes X Boron stress	1.310	4	0.328	1.590	0.185
Genotypes X Hormone types	2.283	1	2.283	11.080	0.001
Boron stress X Hormone types	4.796	4	1199	5818	0.000
Genotypes X Boron stress X Hormone types	1.780	4	0.445	2.160	0.081
Error	16.485	80	0.206		
Total	46.161	99			

Table 4 Analysis of variance of callus weights at 4 mg L⁻¹ 2,4-D/Dicamba in mature wheat embryos

Source	Sum of Squares	df	Mean Square	F	Sig
Genotypes	1.212	1	1.212	5.585	0.021
Boron Stress	8.235	4	2.059	9.486	0.000
Hormone Type	19.740	1	19.740	90.955	0.000
Genotypes X Boron stress	3.382	4	0.846	3.896	0.006
Genotypes X Hormone types	11.868	1	11.868	54.683	0.000
Boron stress X Hormone types	0.776	4	0.194	0.894	0.471
Genotypes X Boron stress X Hormone types	0.917	4	0.229	1.057	0.383
Error	17.363	80	0.217		
Total	61.802	99			

Table 5 Effects of different BAP concentrations under boron stress on plantlets number in mature embryo culture of wheat

Genotypes	Boron concentrations (mg L ⁻¹)	Hormone concentrations (mg L ⁻¹) (BAP)	Plantlets number (n)*			
Einkorn (<i>T. monococcum</i> ssp. <i>monococcum</i>) (IZA)	0	Control (0)	2.13	±	1.03	efgh
		0.5	2.58	±	0.83	def
		2	2.92	±	0.88	cd
	6.2	Control (0)	2.79	±	0.78	cd
		0.5	2.71	±	0.81	cde
		2	2.58	±	0.78	def
	12.4	Control (0)	3.08	±	0.83	bcd
		0.5	2.04	±	0.95	fghi
		2	2.63	±	0.71	cdef
	24.6	Control (0)	1.54	±	0.83	hijk
		0.5	1.92	±	0.83	ghij
		2	1.83	±	1.09	ghijk
	37.2	Control (0)	1.54	±	0.78	hijk
		0.5	1.38	±	0.77	jk
		2	1.33	±	0.70	jk
Bread Wheat (<i>T. aestivum</i> L.) (Cv. Tosunbey)	0	Control (0)	3.17	±	0.92	abcd
		0.5	3.21	±	1.18	abc
		2	3.17	±	0.96	abcd
	6.2	Control (0)	3.67	±	1.13	a
		0.5	3.71	±	1.12	a
		2	3.63	±	1.21	ab
	12.4	Control (0)	3.04	±	0.91	cd
		0.5	2.58	±	0.72	def
		2	2.79	±	0.83	cd
	24.6	Control (0)	1.67	±	0.96	ghijk
		0.5	1.29	±	0.69	k
		2	2.17	±	0.96	efg
	37.2	Control (0)	1.54	±	0.78	hijk
		0.5	1.50	±	0.72	ijk
		2	1.83	±	0.82	ghijk

*Different letters indicate significant differences at $p < 0.05$

affected callus weight in our study. Einkorn wheat resulted in the highest callus weight under 6.2 mg L⁻¹ boron while bread wheat did it under 12.4 mg L⁻¹. Bread wheat was more resistant at high boron concentration than einkorn wheat. Moreover, callus weight decreased when boron concentration increased in the culture media. The study by Huang and Graham (1990) with seven different wheat cultivars under six different boron concentrations on cellular and organ level was also parallel with our research. In our report, only two

and four mg L⁻¹ hormone doses significantly affected callus weight than other hormone doses. In our study, one mg L⁻¹ dicamba containing 6.2 mg L⁻¹ boron for einkorn wheat and four mg L⁻¹ dicamba containing 12.4 mg L⁻¹ boron were the best medium for callus weight. From that point of view, it appeared that dicamba gave better results in wheat mature embryo culture than 2,4-D. This was supported by Murin et al. (2012) with 26 wheat cultivars were cultured on callus media containing dicamba and 2,4-D.

Fig. 2 The effect of 6-Benzylaminopurine (BAP) under the different boron stresses on the plant regeneration from callus derived from mature embryos of einkorn and bread wheat. (A–I: Bread wheat) **A** 0 mg L⁻¹ BAP **B** 0.5 mg L⁻¹ BAP **C** 2 mg L⁻¹ BAP (A, B and C under 0 mg L⁻¹ Boron) **D** 0 mg L⁻¹ BAP **E** 0.5 mg L⁻¹ BAP **F** 2 mg L⁻¹ BAP (D, E and F under 6.2 mg L⁻¹ Boron) **G** 0 mg L⁻¹ BAP **H** 0.5 mg L⁻¹ BAP **I** 2 mg L⁻¹ BAP (G, H and I under 12.4 mg L⁻¹ Boron) **J** 0 mg L⁻¹ BAP with 6.2 mg L⁻¹ boron **K** 2 mg L⁻¹ BAP with 12.4 mg L⁻¹ boron **L** 2 mg L⁻¹ BAP with 6.2 mg L⁻¹ boron **M** Transplanting regenerated einkorn wheat to soil **N–O** Transplanting regenerated einkorn wheat to soil

The most important factor for plantlets number was the amount of boron in culture media. The best plantlets number was the culture media containing 12.4 mg L⁻¹ boron for einkorn wheat and 6.2 mg L⁻¹ boron for bread wheat. Einkorn wheat was more tolerant to boron stress than bread wheat. A previous study on in vitro of pear rootstock under

salt-added boron stress, on the other hand, showed that the number of plantlets decreased with the increased boron and salt amounts in culture media (Sotiropoulos et al. 2006). Genotype-boron stress factor was significant only in culture media containing control group and 0.5 mg L⁻¹ BAP. The best plantlet number in bread wheat was in culture media

Table 6 Analysis of variance of plantlets numbers according to three different BAP concentrations in mature wheat embryos

Source	Sum of Squares	df	Mean Square	F	Sig
A. Analysis of variance of plantlets numbers according to 0 mg L⁻¹ BAP in mature wheat embryos					
Wheat Species	1296	1	1296	0.632	0.427
Boron Stress	1,08,166	4	27,042	13,197	0
Wheat X Boron	1,14,374	4	28,594	13,955	0
Error	20,28,560	990	2049		
Total	22,52,396	999			
B. Analysis of variance of plantlets numbers according to 0.5 mg L⁻¹ BAP in mature wheat embryos					
Wheat Species	0.049	1	0.049	0.025	0.874
Boron Stress	1,27,304	4	31,826	16,439	0
Wheat X Boron	23,836	4	5959	3078	0.016
Error	19,16,690	990	1936		
Total	20,67,879	999			
C. Analysis of variance of plantlets numbers according to 2 mg L⁻¹ BAP in mature wheat embryos					
Wheat Species	0.529	1	0.529	0.308	0.579
Boron Stress	1,94,586	4	48,647	28,283	0
Wheat X Boron	2586	4	0.647	0.376	0.826
Error	17,02,770	990	1720		
Total	19,00,471	999			

containing 0.5 mg L⁻¹ BAP supplemented with 6.2 mg L⁻¹ boron while control group containing 6.2 mg L⁻¹ boron for einkorn wheat. Species did not affect the number of plantlets.

Both wheat genotypes (Einkorn and bread wheat) were transported to the soil. However, bread wheat succeeded the following period better. At the end of 38 days, nodes started to be seen only in bread wheat. At the end of the acclimatization period, flowering (60 days) and ripening (90 days) happened in bread wheat. Einkorn wheat did not form spike. This may be an indication that bread wheat was better under acclimatization conditions.

Conclusions

Up to now, no studies have been reached, indicating efficient tissue culture protocol of einkorn (*Triticum monococcum* ssp. *monococcum*) and bread (*Triticum aestivum*) wheat via using mature embryo under different boron doses. Our study also revealed that hormone, genotype and boron affected both wheat species in vitro. In the present study concluded that callus weight and plantlet regeneration were more tolerant at high concentrations of boron in bread and einkorn wheat, respectively. It is important to point out that this report may help future in vitro studies of different wheat species or crops under boron stress conditions. Moreover, such effective tissue culture protocol may help for wheat improvement programs including breeding, genetic engineering, and genetic transformation.

Fig. 3 Spike development of bread wheat

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Declarations

Conflict of interest No potential conflict of interest was reported by the author(s).

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