BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES

EFFECTS OF 2, 4-D AND DICAMBA ON THE CALLUS PRODUCTION AND REGENERATION OF EINKORN (*TRITICUM MONOCOCCUM* SSP. *MONOCOCCUM*) AND BREAD WHEAT (*TRITICUM AESTIVUM* L.) UNDER BORON TOXICITY

MASTER OF SCIENCE

FERDİ AĞIL

BOLU, JUNE 2020

BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES DEPARTMENT OF BIOLOGY

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APPROVAL OF THE THESIS

Effects of 2, 4-D and Dicamba on The Callus Production and Regeneration of Einkorn (*Triticum monococcum* ssp. *monococcum*) and Bread Wheat (*Triticum aestivum* L.) Under Boron Toxicity submitted by **Ferdi AĞIL** and defended before the below named jury in partial fulfillment of the requirements for the degree of **Master of Science in Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 30/06/2020 by**

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Director of Institute of Graduate Studies

To my now and future family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ferdi AĞIL

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ABSTRACT

EFFECTS OF 2,4-D AND DICAMBA ON THE CALLUS PRODUCTION AND REGENERATION OF EINKORN (*TRITICUM MONOCOCCUM* SSP. *MONOCOCCUM*) AND BREAD WHEAT (*TRITICUM AESTIVUM* L.) UNDER BORON TOXICITY

MSC THESIS

FERDİ AĞIL

BOLU ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF BIOLOGY

(SUPERVISOR: PROF. DR. NUSRET ZENCİRCİ)

BOLU, JUNE 2020

Wheat has been a big part of human history since the first agriculture activity appeared in the past. The first cultivated species, IZA (Einkorn) (*Triticum monococcum* subsp. *monococcum*) is an important part of this historical process. In addition, bread wheat (*Triticum aestivum*), which we use for making bread, has, currently, great importance for human nutrition in the world. Today, biotechnological developments are preferred more than classical methods to increase the nutritional values and quality of these wheat and other plant species. In this study, callus formation was achieved by applying embryo culture under five different doses boron stresses (0, 6.2, 12.4, 24.8, and 37.2 mg/L) on mature embryos of diploid IZA and hexaploid variety of Tosunbey wheat, with four different concentrations (0, 1, 2 and 4 mg/L) of two different auxin hormones (2,4-D and Dicamba). The calli formed were then moved to environments with three different BAP concentrations (0, 0.5, and 2 mg/L) to succeed regeneration. Samples when they reached to a certain length, they were moved to containers that contain the same media for their development. Regenerated certain length samples were moved to magenta containers with the same media for the development. The embryos developed in *in vitro* conditions were allowed to acclimate to the soil after reaching 6-7 cm in length in the magenta boxes. For IZA wheat, the highest and the lowest callus weight was 3.54 and 1.99 g in 2,4-D. media. In Dicamba, 3.71 and 2.02 g were found. For bread wheat, 1.20 and 2.57 g were observed in 2.4-D media while 1.68 and 3.46 g were observed in Dicamba one. Callus dimensions were found as 0.79 and 0.95 cm in 2.4-D for IZA wheat, respectively. It was 0.87 and 1.10 cm in Dicamba. In bread wheat, the results for 2, 4-D are 0.78 cm and 1.01, while in Dicamba it is 0.98 and 1.22 cm. The highest regeneration capacity was 71.33% of IZA wheat and 65.33% for bread wheat. The lowest regeneration capacity was 6% in IZA wheat and 7.33% in bread wheat. The highest number of plants was 3.08 in IZA wheat and 3.71 in bread wheat. The lowest values were 1.33 in IZA wheat and 1.29 in bread wheat. These results showed that IZA wheat is more tolerant to boron stress than bread wheat at the tissue culture level. On the other hand, bread wheat was more effective than IZA for plant regeneration and development.

KEYWORDS: Wheat, Tissue Culture, Embryo, Einkorn, Bread, Boron

ÖZET

BOR TOKSİSİTESİ ALTINDA, 2, 4-D VE DİCAMBA'NIN SİYEZ (*TRİTİCUM* MONOCOCCUM SSP. MONOCOCCUM) VE EKMEKLIK BUĞDAY (*TRİTİCUM AESTİVUM* L.) TÜRLERİNİN KALLUS ÜRETİM VE BİTKİ REJENERASYONUNA ETKİLERİ
YÜKSEK LISANS TEZI
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BIYOLOJI ANABILIM DALI
(TEZ DANIŞMANI: PROF. DR. NUSRET ZENCİRCİ)

BOLU, HAZİRAN – 2020

Buğday, geçmişte tarımın ilk ortaya çıkmasından günümüze kadar insanlık tarihinin önemli bir parçası olmuştur. İlk kültüre alınan tür olan IZA (Einkorn) (*Triticum monococcum* subsp. *monococcum*) bu tarihsel sürecin önemli bir parçasıdır. Ayrıca ekmek yapmak için kullandığımız ekmeklik buğday (*Triticum aestivum*), günümüzde dünyada insan beslenmesi için büyük önem taşımaktadır. Bu çalışmada, diploid ve heksaploid buğday türleri olan IZA ve tescilli Tosunbey buğdaylarının olgun embriyoları iki farklı oksin türünün (2,4-D ve Dicamba) dört farklı konsantrasonu (0, 1, 2 ve 4 mg/L) ile beş farklı doz bor stresi (0, 6.2, 12.4, 24.8 ve 37.2 mg/L) altında embriyo kültürü uygulanarak kallus oluşumu sağlanmıştır. Oluşan kalluslar daha sonra re-jenerasyon sağlamaları için 3 farklı BAP konsantrasonu (0 mg/L, 0.5 mg/L, 2 mg/L) bulunan ortamlara taşınmışlardır. Rejenere ve belli bir uzunluğa erişen örnekler gelişimlerini sağlamaları için aynı besi ortamına sahip magenta kutularına taşınmışlardır. Laboratuvar şartlarında gelişen embriyolar magenta kutularında 6-7 cm uzunluğa ulaştıktan sonra toprağa alıştırılmaları sağlanmıştır. IZA buğdayı için, 2,4-D içeren ortamlarda en yüksek ve düşük kallus ağırlığı 3.54 g ve 1.99 g olmuştur. Dicamba içeren ortamlarda ise 3.71 g ve 2.02 g bulunmuştur. Ekmeklik buğday için, 2,4-D içeren ortamlarda 1.20 g ve 2.57 g olurken Dicamba'da 1.68 g ve 3.46 g gözlemlenmiştir. Kallus boyutları IZA buğdayı için, 2,4-D'de sırasıyla 0.79 cm ve 0.95 cm bulunmuştur. Dicamba için 0.87 cm ve 1.10 cm olmuştur. Ekmeklik buğdayda, 2,4-D için sonuçlar 0.78 cm ve 1.01 iken Dicamba'da 0.98 cm ve 1.22 cm'dir. En yüksek re-jenerasyon kapasitesi sırasıyla IZA buğdayın %71.33 iken ekmeklik buğdayda %65.33 olmuştur. En düşük re-jenerasyon kapasitesi ise IZA buğdayında %6 iken ekmeklik buğdayda %7.33 bulunmuştur. Bitkicik sayısı bakımından en yüksek değer IZA buğdayında 3.08 iken ekmeklik buğdayda 3.71 görülmüştür. En düşük değerler ise yine IZA buğdayında 1.33 iken ekmeklik buğdayda 1.29 olmuştur. Bu sonuçlar IZA buğdayının bor stresine doku kültürü seviyesinde ekmeklik buğdaya göre daha avantajlı olduğunu göstermektedir. Öte yandan bitki oluşması ve gelişmesi açısından ekmeklik buğday IZA buğdayına göre daha etkili olmuştur.

ANAHTAR KELİMELER: Buğday, Doku Kültürü, Embriyo, IZA, Ekmeklik, Bor

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FAO	: Food and Agriculture Organization
TUIK	: The Turkish Statistical Institute
CRIFC	: Field Crops Central Research Institute
PGR	: Plant Growth Regulator
M	: Molarity
N	: Normality
NaOH	**:** Sodium Hydroxide
HCl	: Hydrochloric Acid
H3BO3	: Boric Acid
Dicamba	: 3,6-Dichloro-2-Methoxybenzoic Acid
2,4-D	: 2,4-Dichlorophenoxyacetic Acid
BAP	: 6-Benzylaminopurine
MS	: Murashige and Skoog media
Lx	: Lux
B	: Boron
L	: Liter
Kg	: Kilogram
g	: Gram
mg	: Milligram
ml	: Milliliter
cm	: Centimeter
n	: Number
µ	: Micro
ha	: Hectare
B.C.	: Before Christ
°C	: Celsius
Cult.	: Cultivated

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1. INTRODUCTION

Today, about 3,000 of the 250,000 plant species are used, and 150 species are cultured regularly. Cereals which belongs to the Generae (Gramineae / Poaceae) family are also the first cultivated plants and constitute the product group with the highest cultivation and production on earth. Wheat (*Triticum*), barley (*Hordeum*), rye (*Secale*), oat (*Avena*), triticale (*Triticosecale Wittmack*), corn (*Zea*), paddy (*Oryza*), millet (*Sorghum, Panicum, Seteria*) and bird meal in the cereals group (*Phalaris*) the first four are “Cool Climate Grains”; others are gathered under the name of "Warm Climate Grains" (Kün, 1988).

Due to the wide adaptation ability, high feeding value, easy processing and storage, wheat, which ranks first among other cultivated plants in the world for cultivation and production, decreased the cultivation area to 2.18 billion hectares in 2017 and decreased the production to 771 million tons (FAO, 2019).

Wheat grows in many regions in Turkey and in the world. It is an important plant for its large production mass and the basic nutrient value for people. Throughout history, cereals have been used as basic foodstuffs in larger areas in many cultures with their easy storage and simple processing (Şehirali and Özgen, 2013). Wheat, which has a large cultivation area among cereals, is generally used in bread, pasta, biscuits, bulgur and many food productions. Wheat, which is the main nutrient, addresses about 35% of the world's population (Kün, 1996). It is a plant grown almost anywhere in the world due to its adaptation to different soil and climatic conditions. Today, its agriculture is more technological and its harvest is machine-based brings more growers to wheat agriculture. Due to these features, wheat will remain a strategic plant in the future as it has been since the past to today (Altuhaish, and Yahya, 2014).

The yield of wheat may depend on the adaptation of the varieties to the environmental conditions. For this purpose, yield stability, in some breeding programs, may constitute the breeding purpose of wheat varieties. Breeding purpose of varieties is to resist abiotic and biotic stress conditions and to obtain high yields under unfavorable environmental conditions as well. Abiotic and biotic factors can be considered as the main source of a decrease in yield. Drought, high temperature and disease-pest stresses are important factors affecting wheat yield and, consequently, quality (Güleç et al, 2010). Among those factors, micro and

macro element deficiency or higher existence of toxic substances may cause to decrease in the yield and quality.

Boron (B) toxicity, one of those abovementioned problems, is an important mineral nutrition difficulty that limits plant growth in many parts of the world, especially in arid and semi-arid territory. Compared with areas where boron deficiency is present, although boron rich soils are less common, it is seen that in different regions of the world, vegetable yield decreases more with boron deficiency. For this reason, boron toxicity is one of the leading causes of decrease in yield (Cartwright et al, 1986).

The toxic levels of B, which is one of the absolute essential nutrients for plants to develop normally, is an important problem that limits plant cultivation in many parts of the world (Başalp et al, 2011). Difficulties in classical breeding methods have directed researchers to apply newly developed biotechnological methods. In the development of new wheat varieties, the most important stage for the application of advanced biotechnology techniques and ensuring success is tissue culture. Plant tissue culture is based on the purposeful processing of plant cells and tissues in vitro. The ability of plant cells to be rapidly reproduced by tissue culture techniques and having a totipotent feature increases the importance of tissue culture. In addition to developing new varieties with tissue culture techniques; significant advances have also been made in the protection of endangered plants, in obtaining disease-free plants and in the storage of plant genetic resources. Embryo and callus culture are the two most commonly used tissue culture techniques (Bajaj, 1991).

1.1 Wheat

Wheat is a grain product belonging to the *Poaceae* family, *Triticeae* tribe. It is thought to be the first cultivated crop (Peleg et al, 2011). Wheat has great importance for meeting the food needs of many people in the world. Today, it is accepted as a basic food by people living from the west of Europe to the north of India, from Scandinavia and from Russia to Egypt. It has the use of food with cultural, economic, and historical significance in many countries including Turkey (Zahory and Hopf, 2000).

Wheat spreads to West Asia, Europe and North Africa immediately after the area where it was cultured first that is, it spreads from the Atlantic Ocean to India, from Scandinavia to the Nile valley (Zohary and Hopf, 2000). It reached to Greece in 6,500 BC and Germany in 5,000 BC. (Diamond, 1997). Wheat remains in China were found between 2,600-1,600 BC (Flad et al, 2010). It is thought that the first wheat to the American continent was taken to Mexico by the Spanish in 1529, and in the USA, it was first planted on the island of Elizabeth in 1602, and then spread to other states. Menno's of German origin living in Crimea migrated to USA-Kansas in 1870-71 and took them with Turkish wheat varieties. Some of the varieties obtained from them were given Russian names, since they were brought from Crimea. (Özberk et al, 2016).

In Turkey, wheat residues have been found in archaeological studies in various regions. According to the studies carried out; various wheat remains were found in Aşıklı Höyük, Çayönü, Hacılar, Can Hasan, Çatal Höyük and Erbaba (Harlan, 1995) (Table.1.1).

Table 1.1. Archaeological studies about wheats

Date (B.C.)	Place	Findings
7.500	Aşıklı Höyük	Einkorn, emmer, durum wheat
7.200	Çayönü	Wild einkorn and emmer; domesticated einkorn and emmer
6.750	Hacılar	Wild einkorn and domesticated emmer
6.500	Can Hasan	Wild einkorn; domesticated einkorn and emmer
6.000-5.000	Çatalhöyük	Domesticated einkorn and emmer
6.000-5.000	Erbaba	Domesticated einkorn and emmer

There are important scientists who contribute to the compilation of wheat genetic resources and their development by moving them to different continents and regions. Russian scientists Vavilov and Zhukovski collected more than 10,000 wheat materials from our country between 1925- 50 (Atar, 2017). Among these, Horanek, a summer wheat variety of Hakkari origin, excelled in many cultural varieties in Russia in 1951 (Qualset et al, 1996). By the US scientist Harlan, local

materials were collected in 2121 from Turkey between the years 1948-64 (Atar, 2017). The most important domestic work by a scientist was the first made in 1935 by Mirza Gökgöl in Turkey, 18, 0000 accessions and over 256 different types of wheat cultivars (varieties) were determined in Yeşilköy Seed Center. Wheat breeding activities in Turkey were, though, launched for the first time at the end of 1925 in Eskisehir Seed Improvement Station. (Bektaş et al, 2016).

Wheat is the most cultivated crop in our country as in the world. While wheat is cultivated on an area of 6.8 million ha in our country, this amount constitutes approximately 58% of the total agricultural land (TSI, 2019). Wheat, which has the largest share in cultivation area, is mostly cultivated in the Central Anatolia Region and followed by South East Anatolia and Mediterranean Regions follow this, respectively. Although the Mediterranean Region is in the third place as cultivation area, it is in the first place in the yield obtained by the unit area. In 2017, 771.7 million-ton wheat was produced in the world on 218.5 million decares (FAO, 2019). In Turkey, according to TSI data from 19 million tons of wheat it was produced in 201 (TSI, 2019). Values for other years are shown in Figure .1.1.

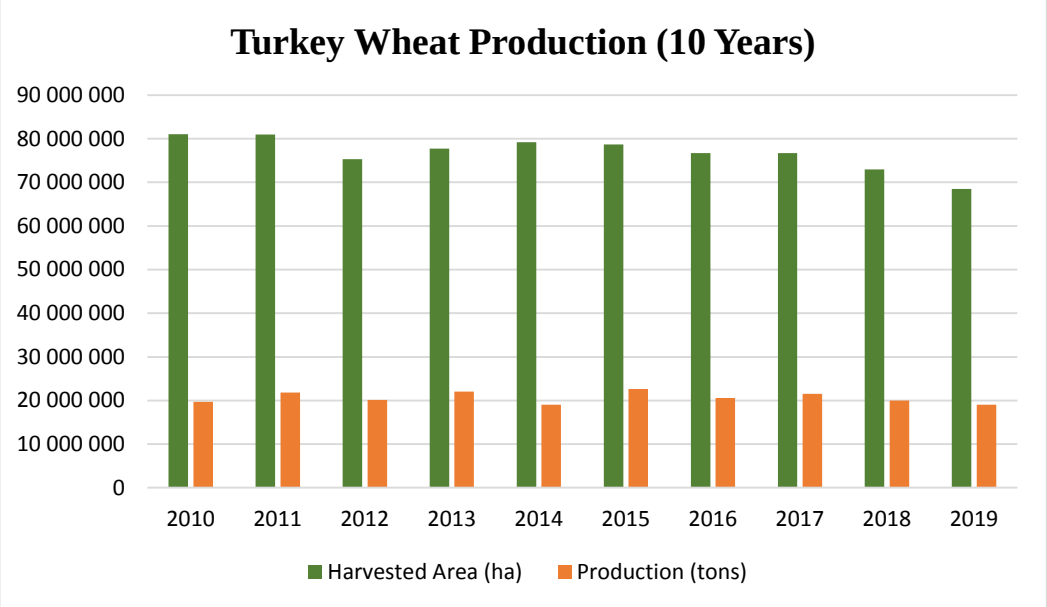

Figure 1.1. Turkey wheat acreage and production in the last 10 years (TSI, 2019).

Wheat, which plays an important role in human nutrition, stands out with its nutritional values. Wheat contains B vitamins, E vitamins, macro-micro elements, sugar, fat, and protein. It also contains 78.1% carbohydrates, 14.7% protein, 2.1%

fat, 2.1 minerals, and 3% B-E vitamins (Shewry et al, 2002; Kumar et al, 2011.) Its nutritional values are shown in table 1.2. (USDA, 2019).

Table 1.2. Nutritional values of wheat (USDA, 2019).

Nutritional value per 100 g Wheat	
Carbohydrates	71.18 g
Fat	1.54 g
Protein	12.61 g
Vitamins	
Thiamine (B1)	0.383 mg
Riboflavin (B2)	0.115 mg
Niacin (B3)	5.464 mg
Pantothenic acid (B5)	0.954 mg
Vitamin B6	0.3 mg
Folate (B9)	38 µg
Vitamin E	1.01 mg
Vitamin K	1.9 µg
Minerals	
Calcium	29 mg
Iron	3.19 mg
Magnesium	126 mg
Manganese	3.985 mg
Phosphorus	288 mg
Potassium	363 mg
Sodium	2 mg
Zinc	2.65 mg

1.1.1 Morphological Structure of Wheat

Cereals belonging to the Gramineae family have common characteristics of the grass plant. Although they have common features, they may differ according to their morphological and composition. This unique family can be improved by

comparing grains such as wheat and corn at maturity. Wheat and other grain types are classified as angiosperm. The flowers have female and male organs and reproduce sexually. Spikes (florets) of cereals such as wheat, rye, and barley are closely connected by rachis (the central backbone of the head) (Jacomet, 2006), (Figure 1.2).

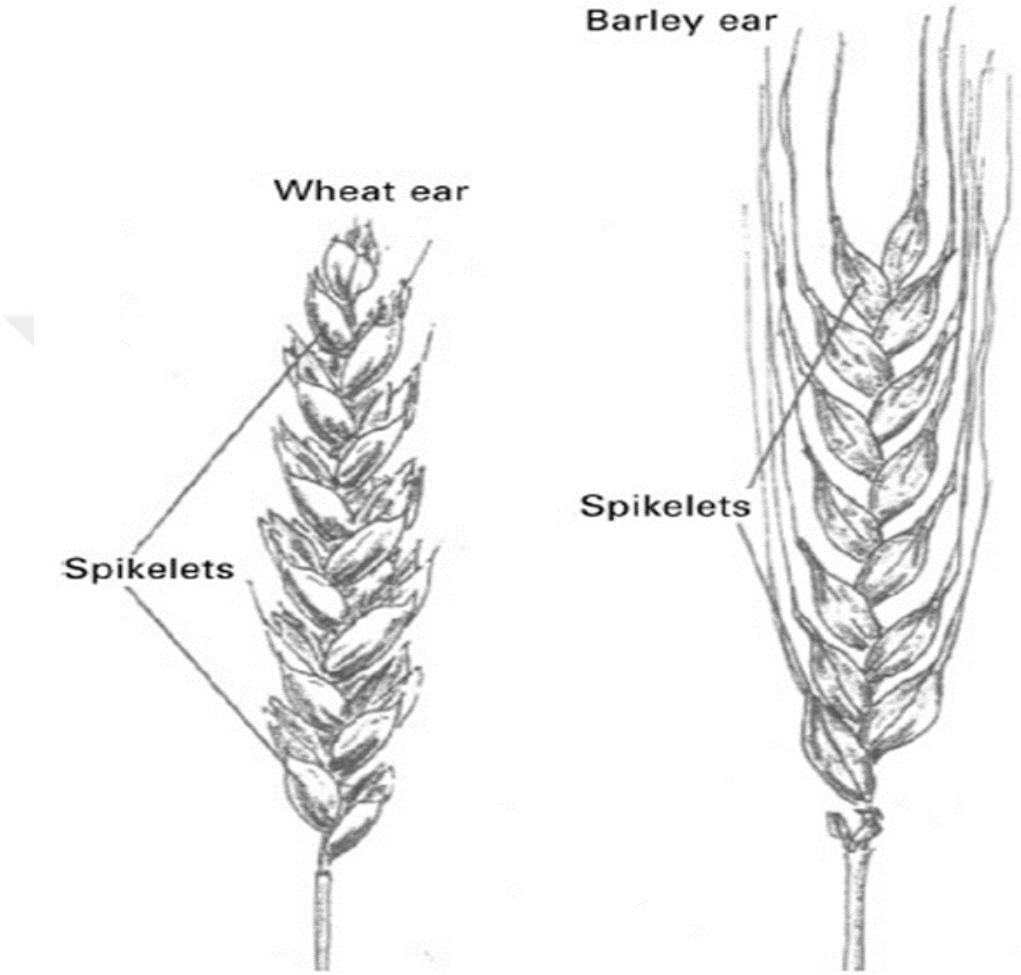

Figure 1.2. The contrasting morphology of seed-bearing head (spike) (Wrigley, 2017, p. 58).

As the stem grows, the grain-bearing head (ear, pointed tip, bunch) develops inside the upper leaf sheath. Finally, the last leaf (flag leaf) appears in the head of light sand and bloom occurs. Wheat is largely self-pollinated, very little exceeded, so pollen is not shared with neighboring flowers. This means that when the genetic structure of the plant stabilizes, it remains true for the genotype during reproduction. The embryo fertilized after flowering develops inside the protective gums. In order to distinguish varieties, it is useful to note the height of the plant at maturity and the strength of straw (both depending on the growing conditions).

A few morphological features of the wheat head are advantageous for identification. The clearest features are the presence or absence of awns (beard), the long hair-like appendage of the lemma glumes, leading to three classes: awnless, half awned, and fully awned. This difference is so clear that it can be seen in a wheat crop when driving past, provided it is enough mature for the heads to be out of the flag leaf. It can be distinguished by using the shapes of the husks (glumes) that surround the seeds in the wheat. In the ripe spike, the glumes dry completely while still wrapping around the grain. The location of the grain is shown in Figure 1.3. In this image, the seed is in the palea and lemma. Lemma continues with the awn out of the spike and up. Each floret of the rachilla has a lemma and a palea, but only the lower florets have the outer glumes. Only these outer glumes are useful for variety identification.

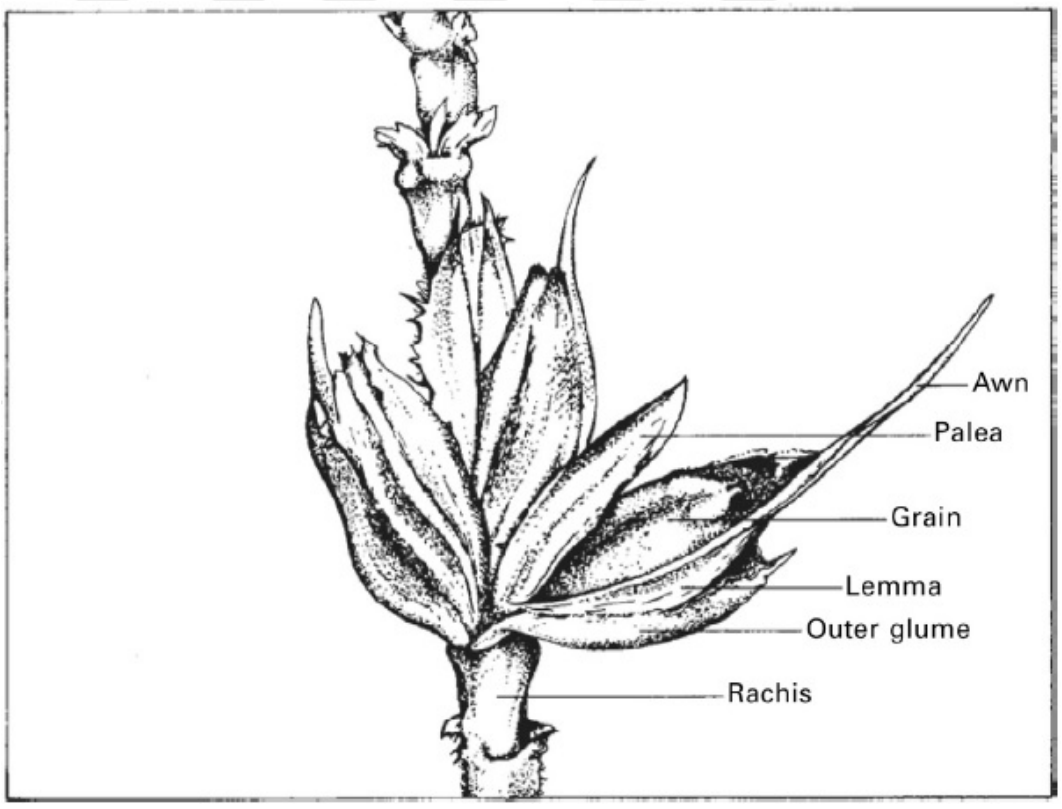

Figure 1.3. Single spikelet of wheat (Wrigley, 2017, p. 64).

Identification of species and varieties is very valuable for grain samples. The first need for this ability is at the early stage of producing pure seeds for sowing. At this stage, authentication can be in the form of checking grain characteristics. Farmers should see that their registered seeds differ from weeds and other wheat. Expertise and experience are required to identify varieties based on grain samples,

and the distinction can never be precise. However, in grain distribution (purchase), if verification of the diversity identity can be carried out immediately after harvest, appropriate delivery volumes can be managed correctly, ensuring that deliveries of different types of cereals are not mixed (Wrigley, 2017).

The best way to understand wheat is to look at its morphological and histological features. Wheat grain is divided into 3 main groups in terms of content: germ, endosperm, and bran (Figure.1.4). Bran forms the outermost layer of wheat; the endosperm is the starchy part under the outer layer. The embryo forms 2-3% of the grain. Includes one embryo and scutellum portion. The embryo differs texturally from the rest of the grain. When grinding wheat, it can be easily decomposed due to this difference. (Heinze, 2017).

The Aleurone layer is a single living cell layer that separates the starchy endosperm and the embryo from the outer layers. It is wealthy in minerals, vitamin B and protein. Even though the aleurone layer is botanically piece of the endosperm, it usually portion from the starchy endosperm with the outer layers during grinding. The outer layers contain the pericarp (inner and outer), the testa and the hyaline layer. They envelope starchy endosperm and germs and guard them from humidity and mold (Posner and Hibbs, 2005).

Most of the grain (81-84%) is starchy endosperm. It is a storage compartment designed as an energy source for the germination of the plant. Energy is stored in endosperm cells as proteins and starch, resulting in a distinctive, very dense structure (Saulnier et al, 2012; Campbell, 2007; Heinze, 2017).

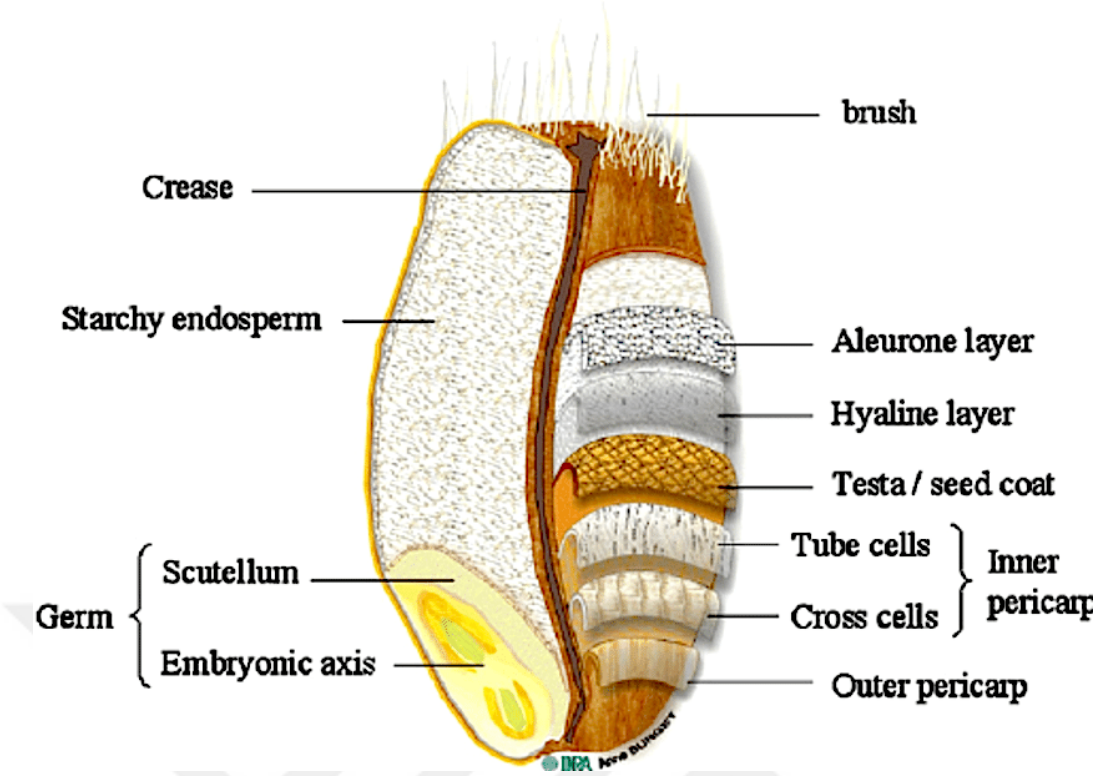

Figure 1.4. Parts of wheat grain (Surget and Barron, 2005).

1.1.2 Origin, Cultivation, and Classification of Wheat

Wheat started to be cultivated in the Fertile Crescent region (Turkey, Iran, Syria, Lebanon, Israel, Palestine and Egypt) approximately 10,000 years ago (Nesbitt and Samuel, 1996) (Figure1.5). According to the studies, it was determined that wheat was first cultivated near the Karacadağ region. There are 23 wild and 400 cultivated varieties of wheat in Anatolia (Özberk et al, 2016). Vavilov, the Russian botanist and geneticist, (1987) determined that two of the eight centers located in Turkey. With the inclusion of wheat, people made the transition from hunter and gatherer to sedentary life. The cultivated wheat is divided into three main groups: diploid (einkorn), tetraploid (emmer, durum, and rivet) and hexaploid (spelled, bread, and club). The first cultivated wheat species were diploid (einkorn) (genome AA) and tetraploid (Emmer) (genome AABB). It indicates that the genetic relationship of these wheat came from the southeastern region of Turkey (Heun et al, 1997; Nesbitt, 1998) The first wheat varieties cultivated by farmers were the ones selected from wild populations, probably because of their superior yields and other characteristics, due to the first and clearly unscientific form of plant breeding. The cultured diploid, tetraploid, and hexaploid wheat forms all have a hard

backbone, apart from the spelled form of bread wheat. Similarly, modern tetraploid and hexaploid wheat forms are free-blended (or naked), while einkorn, emmer, and spelled cultivated forms are all hulled (Shewry, 2009).

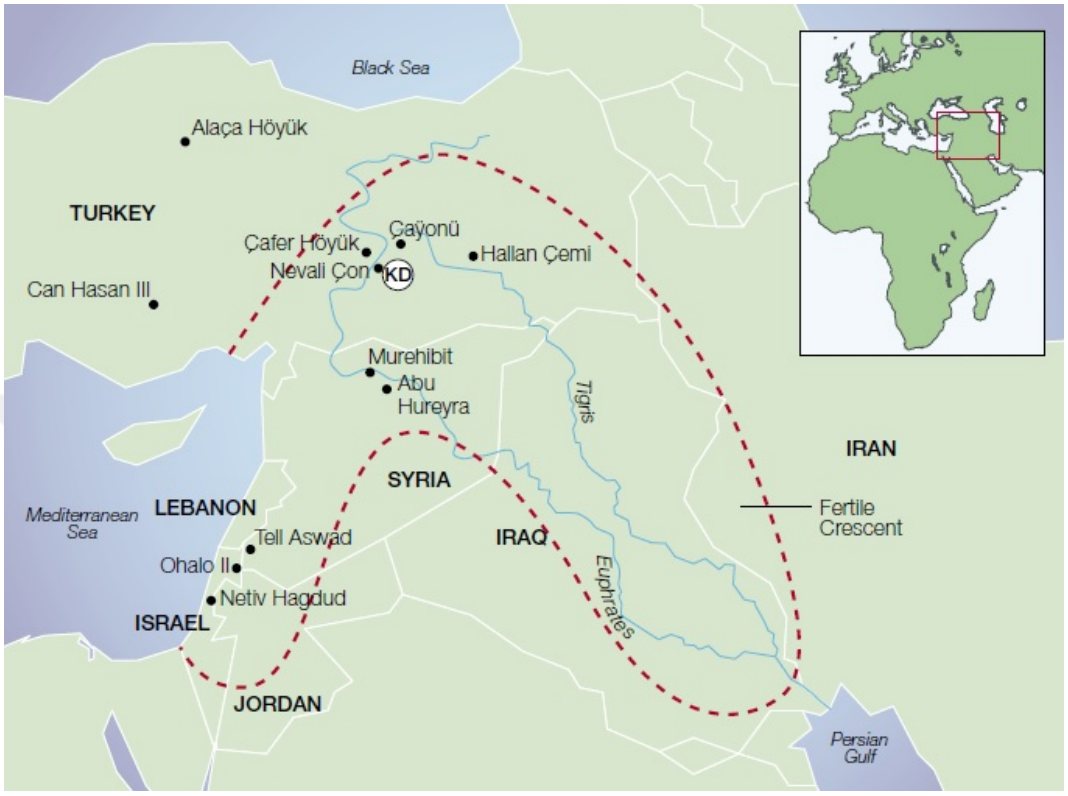

Figure 1.5. Fertile Crescent (Salamini et al, 2002)

The stages of wheat from wild ancestors to the ones we use today are given in Figure 1.6. The first cultivated wheats had a glume, brittle spike. During the maturation period, the spike axis was broken and the spikelets were separated (Zohary and Hopf, 2000). Today, hexaploid bread wheat, *T. aestivum* L. (2n = 42, AABBDD) and *T. durum* Desf. of tetraploid hard or durum wheat. (2n = 28, AABB) is widely produced. It is assumed that the genome A comes from the *T. urartu* Thumanjan *ex* Gandilyan (Dvorak et al, 1998), the genome B comes from Ae. speltoides Tausch (Khlestkina & Salina, 2001), and the genome D from Ae. tauschii Coss (Nesbitt and Samuel, 1998). Today, hexaploid bread *T. aestivum* L. (2n = 42, AABBDD) and tetraploid hard durum *T. durum* Desf. (2n = 28, AABB) wheat are widely produced and mostly bread wheat (*Triticum aestivum* L.) is produced. These varieties have high nitrogen response index and yields. Landraces have high adaptability, drought-heat resistance and higher quality. Despite these features, the reason for the low cultivation in our country is the low yield. Besides, the low

nitrogen response and long length of these varieties affect the cultivation (Özberk et al, 2010).

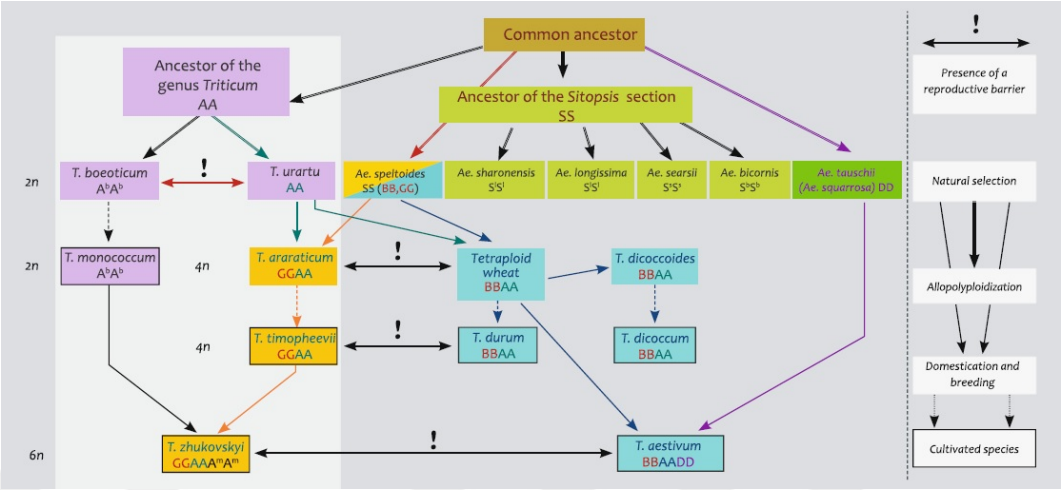

Figure 1.6. Phylogenetic scheme of *Triticum* and Aegilops evolution (Goncharov, 2011)

Wheat is generally classified into 3 main groups. It is classified according to, the growing season (winter or spring wheat), gluten content (hard or soft wheat) and grain color (red, white or amber). In addition, genetic and traditional classifications have been made. Genetic classification was first started by Bowden in 1959 (Bowden, 1959). On the other hand, there are those who use traditional classification. They mostly treat them as Latin binomas. Today, if a genetic classification is preferred, the GRIN classification based on the work of van Slager can be used. If traditional classification is desired, Dorofeev's scheme can be chosen. According to Dorofeev ploidy levels, wheat was classified as diploid, tetraploid, and hexaploidy (Dorofeev et al, 1979; Goncharov, 2011).

1.1.2.1 Diploids, Tetraploids and Hexaploids

Today, wheat is divided into 3 groups according to cytogenetic and cytological studies. Each group has 14 chromosomes (7 pairs) or multiple of 14 chromosomes in their somatic cells. Wheat in diploid group (*Triticum monococcum* subsp. *monococcum* or einkorn) has 14 chromosomes; wheats in the tetraploid group (*Triticum dicoccum* or emmer wheat and *T. turgidum* subsp. *durum* or durum

wheat) has 28 chromosomes and wheat in the hexaploid group (*T. aestivum* subsp. *aestivum* or bread wheat) has 42 chromosomes.

In the archaeological studies, einkorn wheat was determined to be the first cultivated wheat (Cooper, 2015). Einkorn (2n=2x=14), occurs with three different cultivars: wild *T. urartu* Tum. ex Gand. (genome AuAu) and *T. boeoticum* Boiss. (AbAb), and cultivated *T. monococcum* L. (AmAm). In Tetraploid and hexaploid wheat, *T. urartu* is accepted as the A genome ancestor (Brandolini et al, 2015).

Tetraploid species (2n = 4x = 28) can have two different genome formulas with AAGG and AABB genomes. Accordingly, there are 2 polyploid speices: *T. turgidum* progeny (consisting of *T. turgidum* and *T. aestivum*) and the *T. timopheevii* progeny (consisting of *T. timopheevii* and *T. zhukovskyi*). *T. timopheevii* has a limited distribution. Not much work has been done on this wheat. In contrast, *T. turgidum*, which is another one, has a wide production area today. By cultivating wild emmer wheat (*T. dicoccoides*), T. turgidum wheat varieties started to be created. Emmer wheat is also commonly found in the Fertile Crescent region like einkorn wheat (Matsuoka, 2011).

Hexaploid wheats (2n = 6x = 42) are formed by the combination of 3 different genomes (AABBDD). Bread wheat (*T. aestivum*), which is the most grown wheat species today, is the hexaploid form of free-treshing (or naked) wheat. It is thought to consist of hybridization between an allotetraploid wheat (AABB) and diploid (DD) *Ae. tauschii* var. *strangulata*. (Nesbitt and Samuel, 1998; Edwards, 2010) (Table.1.3).

Table 1.3. Ploidy of *Triticum* species (Dvorak, 2001).

Ploidy	Status	Spike	Species	Subspecies
2x	Wild	Hulled, birttle	*T. monococcum*	*aegilopoides*
	Cult.	Hulled, nonbirttle	*T. monococcum*	*monococcum*
	Wild	Hulled, birttle	*T. urartu*	
4x	Wild	Hulled, birttle	*T. turgidum*	*dicocoides*
	Cult.	Hulled, nonbirttle	*T. turgidum*	*dicoccon*
	Cult.	Naked, nonbirttle	*T. turgidum*	*durum, turgidum*
	Wild	Hulled, birttle	*T. tomopheevii*	*armeniaeum*
	Cult.	Hulled, nonbirttle	*T. tomopheevii*	*tomopheevii*
6x	Cult.	Hulled, birttle	*T. aestivum*	*macha, tibetanum*
	Cult.	Hulled, partially birttle	*T. aestivum*	*spelta, vavilovi, yunanense*
	Cult.	Naked, nonbirttle	*T. aestivum*	*aestivum, compactum, sphaerococcum, petropavlovskyi*
	Cult.	Hulled, nonbirttle	*T. zhukovskyi*	

1.2 Tissue Culture

Today, many methods are used in plant breeding, and every day, new varieties with new features contribute to yield and quality increase. The breeding methods used are under two main headings: classical and biotechnological. As an example of classical methods; selection, hybridization, polyploidy, and introductions can be given. The methods used have their own problems. Varieties cannot be improved without adequate adaptation attempts at introduction. In selection studies, it takes a long time for breeding (Şehirali and Özgen, 1998).

With the developing technology in recent years, new breeding methods have been used. These methods add and remove some new features that will improve yield and quality in plants. Along with classical breeding methods, tissue culture

and genetic engineering studies are carried out by using biotechnological techniques for the future (Hancı and Cebeci, 2012).

Plant cell and tissue cultures are techniques in which plant cell tissue (meristematic cells, cell suspension, or callus cells), organs (apical meristem, stem, stem, leaf, etc.) or plantlets are produced under aseptic conditions and in appropriate nutrient media (Babaoğlu et al, 2001). In addition to the ability of plants to reproduce sexually in tissue culture studies, their ability to grow asexually (totipotensi) from any part such as stem, branch and leaf made it possible for them to develop *in vitro* (Stasolla and Thorpe, 2011).

In order to overcome the problems experienced in the classical breeding of wheat and other cereals, it is necessary to benefit from tissue culture and biotechnological methods. Success is dependent on many factors in embryo culture, callus formation, and plant regeneration, which is an important step in gene transfer by applying genetic engineering studies. These are the genotype (Li et al, 2003), plant growth conditions (Hess and Carman, 1998), culture media (Fennel et al, 1996), and explant source (Redway et al, 1990; Aydın et al, 2011).

As an explant source for the formation of somatic callus in wheat, immature embryos, mature embryos (Ozgen et al, 1996; Sarker et al, 2007), leaves (Haliloglu, 2006; Li et al, 2009), mesocotyls (Yurkova et al, 1982), seeds (Aydin et al, 2011), apical meristems (McHughen, 1983), shoot tips (Aguado-Santacruz et al, 2011) and anthers (Delporte et al, 2001) are used. Callus culture is the encouragement of different organ tissues of plants to form callus (undifferentiated cell stack). The callus may be embryogenic or not. However, embryogenic callus is important for plant regeneration from callus tissue (Bürün, 1996), (Figure 1.7).

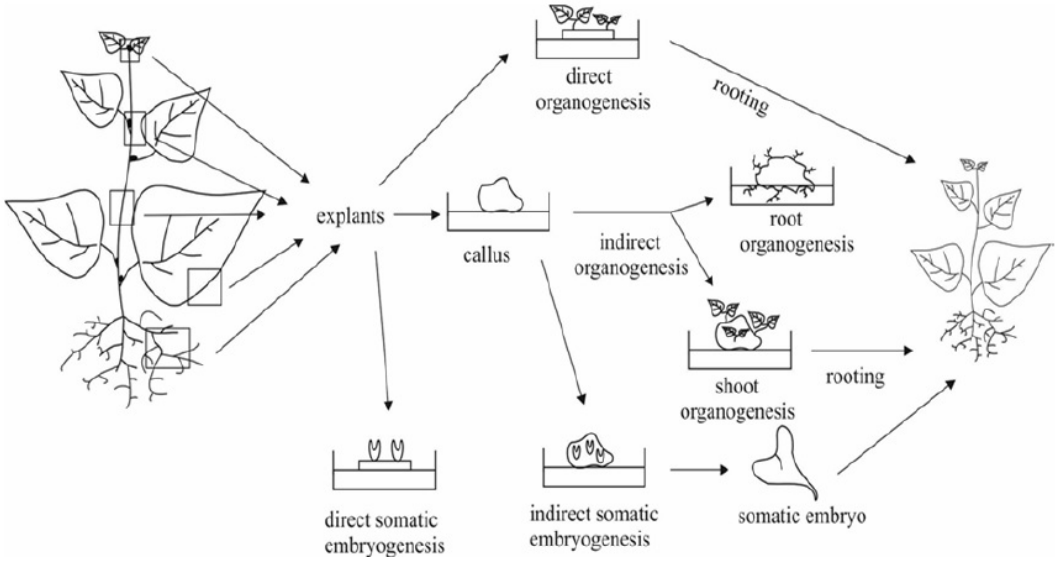

Figure 1.7. Plant regeneration stages in tissue culture (Adamczuk et al, 2012).

It is the most frequently used immature embryo that gives the best results among the explant varieties used for callus culture. However, the ability to obtain immature embryos at a certain time of the year has a negative effect on explant selection. There is no such restriction for the use of mature embryos. Unlike immature embryos, the physiological state of mature embryos does not differ much. The seeds obtained can be stored in different places for future use (Delporte et al, 2001).

1.3 Boron Toxicity

Boron is an important underground resource with strategic importance for the world and our country. Turkey has 64% of the world's boron reserves (Doğan et al, 2005). Boron is an important micro element for plants. In its deficiency, there is a decrease in growth, yield and quality in plants. When it is found in large amounts in the soil, it causes toxicity in plants. Boron toxicity is an abiotic stress factor observed especially in arid and semi-arid regions of our country (Kalaycı et al, 1998). In a study, it was stated that the highest boron amount was in the Central Anatolia Region and the lowest boron amount was in the Black Sea, Aegean and Marmara regions (Sillanpaa, 1982).

The source of the amount of boron in the soil is groundwater and naturally occurring borders. The amount of boron in the soil is increased by fertilization and irrigation (Nable et al, 1997). Boron toxicity and deficiency have a significant effect on plant grain yield. Boron levels that cause boron toxicity and deficiency contain very close values (Chapman et al, 1997). For this reason, boron deficiency and toxicity symptoms are among the most common stress factors among micronutrients (Taner et al, 2003).

As a result of excessive boron accumulation in the soil, a micro element problem occurs that prevents the root and shoot growth of the plants and limits the grain yield. It is stated that especially in barley grains, barley and wheat are sensitive to excess boron in the soil (Gupta et al, 1985). For this reason, there is a significant decrease in their productivity due to excess boron in grain types and varieties that are sensitive to boron (Cartwright et al, 1986). Excess boron causes adverse effects on most developmental and biochemical pathways in plants such as photosynthesis, stem cell division, enlargement of the exile cell wall and deoxidative damage with the increase of reactive oxygen species (Cervilla et al, 2007)

Boron is an indispensable food for many organisms. Plants take boron from the soil in boric acid form. Boron taken from the roots is carried to the shoots by xylems. The boron carried along the long distance follows the sweating current and accumulates on the edges of the mature leaves (Brown and Shelp, 1997). Besides its boron toxicity, its deficiency is also an important problem in many parts of the world. Therefore, an optimum amount of boron is required for normal plant development. In areas with boron deficiency, boron is given as fertilizer to solve this problem, but attention should be paid. Because giving too much causes toxicity (Takano et al, 2008)

2. AIM AND SCOPE OF THE STUDY

Boron toxicity is an important problem that can limit plant growth in soils under arid and semi-arid environmental conditions. High boron concentrations may occur naturally in soil or groundwater, or may be mixed with the soil by mining, fertilizer or irrigation water. The amount of boron that plants need is quite low. Although the amount of boron needed is very small, this amount stops the development of wheat. Plants such as wheat and barley contain 20-70 mg / kg boron. As in plant species, boron contents can vary between genotypes within the same species.

Boron deficiency especially affects the active growth points in the root and green parts. Boron is one of the most difficult nutrients in the phloem channel in most plant species (i.e. moving to young leaves and active growth points within the plant). Therefore, in plants where boron mobility is very low, boron deficiency occurs first in the youngest parts of plants, root growth tips, and especially in fruits. Shallow deformations and yellowing may occur on young leaves. Flowering and fertilization in boron deficiency in wheat results in significant decreases and the seed production capacity of plants decreases. Turkey is a country rich in wheat production and genetic diversity. In 2019, 11.9 million hectares of grain were planted in our country. 6.8 million hectares of wheat were planted in the cultivated grain field and 19 tons of wheat were produced (TSI 2019). However, it is very important to produce wheat sufficient for this rapidly increasing population. For this purpose, intensive wheat breeding studies are carried out and agricultural biotechnology is also used extensively to make this more effective. Therefore; *in vitro* cultivation techniques, appropriate tissue culture and protocols are being developed. Thus, the aim of this study was

1. To succeed embryo culture under boron stress,
2. To determine and compare of callus induction and regeneration rate with different auxins and concentrations,
3. To determine the best callus and regeneration rate,
4. To develop some appropriate protocols

3. MATERIALS AND METHODS

The experiments were conducted at the Laboratory of Plant Genetics & Pathology, Department of Biology, University of Bolu Abant Izzet Baysal University, Bolu, Turkey.

3.1 Materials

3.1.1 Plant Sources

In this study, one einkorn (IZA: *Triticum monococcum* subsp. *monococcum*) wheat from 2016 Seben / Bolu harvest and one bread wheat (*Triticum aestivum* cultivar: Tosunbey) were studied (Figure 3.1). Bread wheat cultivar was provided by Central Research Institute for Field Crops (CRIFC).

Figure 3.1. Wheat's grains. **A**: Einkorn (*T. monococcum* subsp. *monococcum*). **B:** Bread (*T. aestivum*).

3.1.2 Chemicals

3.1.2.1 Chemicals for Sterilization

For sterilization, sterile distilled water, Tween20, 70 % ethyl alcohol, and 1% sodium hypochlorite were used.

3.1.2.2 Chemicals for Callus Induction and Plant Regeneration Media

In plant growth media, in callus culture, and plant regeneration media Murashige and Skoog (1962) were used as the main nutrition media. Agar and

sucrose were also, used. Agar (Duchefa) was used as a gel constructor. Sucrose (Duchefa) was added in addition to MS (Duchefa) and agar. For boron-free media, micro-macro elements and vitamin contents in MS media were re-prepared according to Murashige and Skoog (1962), but Boric acid was not used. The chemicals used in the callus and regeneration stage are shown in Table 3. 1.

Table 3.1. Callus Induction and Plant Regeneration Media Contents (Murashige and Skoog, 1962).

Micro Elements	**mg/L**
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
Na_2.EDTA	37.26
$Fe_2(SO_4).7H_2O$	27.8
H_3BO_3	6.20
KI	0.83
$MnSO_4.H_2O$	16.90
$Na_2MoO_4.2H_2O$	0.25
$ZnSO_4.7H_2O$	8.60
Macro Elements	
$CaCl_2$	332.02
KH_2PO_4	170.00
KNO_3	1900.00
$MgSO_4$	180.54
NH_4NO_3	1650.00
Vitamins	
Glycine	2.00
myo-Inositol	100.00
Nicotinic acid	0.50
Nicotinic acid	0.50
Thiamine HCl	0.10
Total	**4.4**
Sucrose	30.000
Agar	8.000

3.1.2.3 Chemical of Plant Growth Regulators

In this study different types of auxins; 2, 4-D (2, 4-Dichlorophenoxyacetic acid) and Dicamba (3, 6-Dichloro-2-methoxybenzoic acid) and cytokinin; BAP (6-Benzylaminopurine) were used.

3.2 Methods

The methods used in the research are described in detail under separate titles, including sterilization, culture techniques, acclimatization and data evaluation.

3.2.1 Sterilization

Sterilization of the laminar air-flow cabin (Nuve- LN 090), materials and explants to be used in the cabinet were performed. The sterile laminar air-flow cabin was rinsed with ethyl alcohol prior to each run and run empty for 10 minutes. Sterilization applied in the study, equipment and explants are explained below.

3.2.1.1 Sterilization of Glass Wares and Instruments

In vitro studies, the sterility of the laboratory and all equipment used are of great importance for successful results without bacterial contamination. Therefore, the equipment was sterilized before each study. For this reason, in this study, beakers, forceps, strainers, scalpels, spoons, glass petri dishes and magenta boxes were sterilized in an autoclave (Nuve- OT 40L) at 121 °C for 15 minutes. The scalpel and forceps used to separate mature embryos from the endosperm and other culture procedures in a laminar air-flow cabinet were sterilized using 70% (v/v) ethyl alcohol and direct flame.

3.2.1.2 Preparation of Sterile Distillated Water

Chlorine in tap water has toxic effects and prevents the development of tissue culture. Distilled water is the most commonly used substance in laboratory studies. Distilled water has been used in laboratory studies such as in the preparation of media and the sterilization of seeds. Distilled water is filled into closed glass containers and made ready for autoclaving. In the autoclave based on the principle of sterilization with water vapor pressure, the glass containers were kept loosely at 121 °C for 15 minutes and sterilized distilled water was obtained.

3.2.1.3 Sterilization of Seeds

The seeds were separated from the chaffs before sterilization. Then, for seed sterilization, einkorn and bread wheat seeds were put into 100 ml distilled water contain a few drops of Tween 20 (Merck) and, then, were mixed for one minute using a magnetic stirrer. After one minute, seeds were washed with sterile water. Then, all seeds were put into 40% commercial bleach (4.6 %15 NaClO) and mixed for 15 min using a magnetic stirrer. After 15 minutes, seeds were washed with sterile water until removing (Örgeç et al, 2018).

At the end of surface sterilization, the seeds were placed in a glass beaker in a sterile cabinet for easy embryo extraction and sterile distilled water was added. They were sealed with aluminum paper and kept in a hot water bath in sterile pure water at 33 ° C for approximately 2 hours (Özgen et al, 1998).

3.2.2 Preparation of Culture Media

Throughout this research, MS media containing various hormone concentrations and combinations was used as the culture media. Sucrose was used as the carbon source. The pH of the prepared media was adjusted at 5.7-5.8 using 1 M NaOH and 1 M HCl. Agar was used to solidify the prepared culture media. The stock solutions of the auxins and cytokinins used were primarily prepared for the culture media. The chemicals were used as solvents and diluents in preparation of stock solutions of auxins and cytokinins,

Table 3.2. Solvents, diluents and storage conditions of auxin and cytokinins. storage conditions

Plant Growth Regulator	Solvent	Diluent	Storage Condition
Auxin			
2,4-D (Sigma)	Ethanol or 1 N NaOH	Water	Room temp.
Dicamba (Duchefa)	Ethanol or Acetone	Water	Room temp.
Cytokinins			
BAP (Sigma)	1 N NaOH	Water	Room temp.

To prepare a 1 mg/ml stock solution: Add 100 mg of the plant growth regulator to a 100 ml volumetric flask or another glass container. Add 2 - 5 ml of solvent to dissolve the powder. After complete dissolution, complete 100 ml with distilled water. The stock solutions were stored in a refrigerator at +4 °C. Add 1.0 ml of the stock solution to 1 liter of media to obtain a final concentration of 1.0 mg/L of the plant growth regulator in the culture media.

3.2.2.1 Preparation of MS Stock Solution without Boron

Macro and micro elements and vitamins that should be present in the nutrient environment (Table 3.2) in the preparation of stock solutions were added in the following order. Boric acid (H_3BO_3) was not used in these elements.

For micro elements,

- Except for H_3BO_3, $FeSO_4.7H_2O$, and Na_2EDTA, 100 times the amount of micro elements required in 1 liter of nutrient media were weighed at a sensitive balance.
- The weighed microelements were added respectively to a 2-liter beaker placed on a magnetic stirrer.
- 600 ml of distilled water were added to dissolve the substances in the beaker.
- After the added microelements were dissolved well in water, the volume of the mixture was completed to one liter.

- A label indicating the preparation date, content and concentration (100 X) of the solution was affixed to the vial.
- It was placed in the refrigerator (+ 4 ℃) for storage.

For macro elements,

- The amount of macro elements required in 1 liter of media was weighed 10 times with a sensitive balance.
- The weighed microelements were added respectively to a 2-liter beaker placed on a magnetic stirrer.
- 600 ml of distilled water were added to dissolve the substances in the beaker.
- After the added microelements were dissolved well in water, the volume of the mixture was completed to one liter.
- A label indicating the preparation date, content and concentration (10 X) of the solution was affixed to the vial.
- It was placed in the refrigerator (+ 4 ℃) for storage.

For $FeSO_4.7H_2O$ and Na_2EDTA,

- 2.78 g $FeSO_4.7H_2O$ and 3.726 g Na_2-EDTA, which are 100 times the amount of $FeSO_4.7H_2O$ and Na_2-EDTA, which are required to be present in one liter of media, were weighed on a sensitive balance.
- 3.726 g of Na_2-EDTA was dissolved by heating added to a 1-liter Erlenmeyer flask containing 350 ml of distilled water and placed on a magnetic stirrer. Again, in a separate vessel, 2.78 g $FeSO_4.7H_2O$ was dissolved in 350 ml of distilled water by heating.
- These two separate solutions were combined to complete the total volume to 1 liter. After this coupling, the color of this solution was light yellow. This prepared solution was transferred to an amber bottle and stored in a refrigerator (4 ° C) so as to avoid direct sunlight.

For vitamins,

- 100 times the amount of vitamins required in one liter of nutrient media was calculated for a 100 ml stock solution and weighed on a sensitive balance.

- The weighed vitamins were added sequentially to a 250 ml Erlenmeyer container containing 30 ml of distilled water and placed on a magnetic stirrer.
- After the added vitamins were thoroughly dissolved in water, the volume of the mixture was completed to 100 ml.
- After the preparation date and concentration were written on the prepared vitamin solution, it was stored in a glass bottle (4 °C) in the refrigerator.

3.2.2.2 Preparation of Callus Induction Medium without Boron

Since media are prepared in very small quantities in the preparation of media, it is problematic to prepare them each time. For this purpose, stock solutions of certain densities which are easier to use are prepared or ready-made media are used. In this study, MS boron-free media was used.

For preparation callus induction media:

- 100 ml of MS macro element stock solution (10X) and 10 ml of micro element stock solution (100X) were added to a 1-liter graduated cylinder.
- On the macro and micro elements, 10 ml of FeSO4.7H2O and Na2EDTA stock solution were added and mixed until dissolved.
- 30 g/L sucrose and 750 ml of distilled water were added to the graduated cylinders. The flasks were placed with a magnetic stir bar and mixed with a magnetic stirrer until dissolved.
- Distilled water was added to complete the whole solution for 1 liter.
- Two liters of solution is divided into 10 different 200 ml autoclave bottles.
- Different concentrations of 2, 4-D (0, 1, 2, and 4 mg/L) and Dicamba (0, 1, 2, and 4 mg/L) were added to each flask separately.
- The pH of each callus formation media was adjusted at 5.7-5.8.
- 1.5 g/L agar was added to each 200 ml solution bottles.
- The bottles were half-closed and placed in an autoclave at 120 ° C for 15 minutes for sterilization.
- After sterilization, the solutions were transferred to laminar air flow and poured into the petri dish (each petri dish had about 40 ml solution).

- In the laminar air-flow cabinet, the media which were allowed to cool and solidify were kept for transport of embryos.

3.2.2.3 Preparation of Callus Induction Media with Normal Boron

For preparation callus induction media:

- 4.4 g/L of MS was weighed and placed on two 1-liter graduated cylinder.
- 30 g / L sucrose and 750 ml of distilled water were added to the graduated cylinders. The flasks were placed with a magnetic stir bar and mixed with a magnetic stirrer until dissolved.
- Distilled water was added to complete the whole solution for 1 liter,
- Two liters of solution is divided into 10 different 200 ml autoclave bottles.
- Different concentrations of 2,4-D (0, 1, 2, and 4 mg/L) and Dicamba (0, 1, 2, and 4 mg/L) were added to each flask separately.
- The pH of each callus formation media was adjusted to 5.7-5.8.
- 1.5 g/L agar was added to each 200 ml solution bottles.
- The bottles were half-closed and placed in an autoclave at 120 °C for 15 minutes for sterilization.
- After sterilization, the solutions were transferred to laminar air flow and poured into the petri dish (each petri dish had about 40 ml solution).
- In the laminar air-flow cabinet, the media which were allowed to cool and solidify were kept for the transport of embryos.

3.2.2.4 Preparation of Callus Induction Medium with Boron

Boron amount can increase according to media except boron-free (0 mg/L boron) and normal (6.2 mg/L boron) media. For callus induction media with boron has the same procedure as the first. After the addition of MS and sucrose, separate boron media is prepared for different boron levels. Accordingly, media containing 12.4 mg/L, 24.8 mg/L and 37.2 mg/L boron were prepared.

For preparation callus induction media;

- For different boron media 4.4 g/L of MS was weighed and placed on two 1-liter graduated cylinders.
- 30 g / L sucrose and different boron doses than 750 ml of distilled water were added to the graduated cylinders. The flasks were placed with a magnetic stir bar and mixed with a magnetic stirrer until dissolved.
- Distilled water was added to complete the whole solution for 1 liter.
- Two liters of solution is divided into 10 different 200 ml autoclave bottles.
- Different concentrations of 2,4-D (0, 1, 2, and 4 mg/L) and Dicamba (0, 1, 2, and 4 mg/L) were added to each flask separately.
- The pH of each callus formation media was adjusted at 5.7-5.8.
- 1.5 g/L agar was added to each 200 ml solution bottles.
- The bottles were half-closed and placed in an autoclave at 120 °C for 15 minutes for sterilization.
- After sterilization, the solutions were transferred to laminar air flow and poured into the petri dish (each petri dish had about 40 ml solution).
- In the laminar air-flow cabinet, the media which are allowed to cool and solidify are kept for transport of embryos.

3.2.2.5 Transfer of Embryos to the Media

The embryos were removed from the mature seeds and taken into prepared MS media in sterile cabinet and under sterile conditions. It is a prerequisite for callus formation that the embryos are carefully removed from the seeds that are kept in the water bath for 2 hours and taken to the prepared media.

- The embryos were removed from the sterile and endosperm softened seeds with the help of scalpel and forceps and placed into petri with the scutellum upwards. Five embryos were placed in each petri and 5 replicates were made for each dose of 2, 4-D and Dicamba.
- The covers of the petri dishes were sealed and wrapped around the film with parafilm. The mature embryos prepared in this way were kept for 28-30 days at 25±1° C under dark conditions in the air-conditioned room with adjustable light and temperature.

- Callus weight, diameter, and formation percentage of calli formed after 28-30 days were obtained. After obtaining the data, the appropriate calli were transferred to the regeneration media.

3.2.2.6 Preparing Plant Regeneration Media

For preparation callus induction media;

- 4.4 g/L of MS was weighed and placed on 1-liter graduated cylinder.
- 30 g/L sucrose and 750 ml of distilled water were added to the graduated cylinders. The flasks were placed with a magnetic stir bar and mixed with a magnetic stirrer until dissolved.
- Distilled water was added to complete the whole solution for 1 liter.
- Two liters of solution was divided into five different 200 ml autoclave bottles.
- Different concentrations of BAP (0, 0.5, and 2 mg/L) were added to each flask separately.
- The pH of each callus formation media was adjusted to 5.7-5.8,
- 1.5 g/L agar was added to each 200 ml solution bottles.
- The bottles were half-closed and placed in an autoclave at 120 ° C for 15 minutes for sterilization.
- After sterilization, the solutions were transferred to laminar air flow and poured into the petri dish (each petri dish had about 40 ml solution).
- In the laminar air-flow cabinet, the media which were allowed to cool and solidify were kept for the transport of calli (Özgen et al. 1998).

3.2.2.6 Transfer of Calli to the Regeneration Medium

In order to provide root and shoot formation from calli obtained from mature embryos, calli were taken to regeneration media containing different BAP concentrations under boron stress.

- The calli obtained by applying 2,4-D and Dicamba under boron stress were taken to the regeneration media containing BAP which was prepared for providing shoot and root growth. For samples that regenerate and reach a certain diameter, media were prepared in magenta boxes with the same content.
- Regeneration was achieved by placing in the culture chamber for 4 weeks for 16 hours in bright 8 hours in dark photoperiod (1500 Lux) and 25 ° C.

- At the end of 4 weeks, regenerated calli in petri were counted to determine regeneration capacity and culture effect.
- The ratio of the regenerated calli to the formed calli was calculated as regeneration capacity (number of regenerated callus / number of formed callus x 100) and the ratio of the regenerated calli to the number of cultured samples was calculated as the culture effect (number of regenerated callus / number of cultured explants x 100).

3.2.3 Acclimatization

At the end of callus formation; there is another important step in plants reaching 10-15 cm height in magenta containers at 16/8 hours light/dark photoperiod and 25 ± 1 ° C temperature in the culture room. Starting from surface sterilization of these plants, they are taken from artificial and sterile environments and transferred to grounded pots to ensure their survival.

Since the plants in the culture are difficult to transfer to the soil and survive, it is necessary to acclimate the samples with an intermediate period before transferring them to the greenhouse or field. When plants are grown in vitro conditions, such as stomata openness, the number of stomata per unit area is too high and the absence of waxy structure in the cuticular layer, there is excessive water loss when taken under normal conditions. In order to prevent this, when the plant is taken to normal environment, it is acclimated during an extremely humid transition period (Pospóšilová et al, 1999; Hazarika et al, 2006).

Therefore, the plantlets that were to be transferred to the climate chamber were carried to the pots consisting of a mixture of vermiculite and peat soil at a ratio of 1:3 and grown at 25 ± 1 °C for a period of 30 days in a 16-hour photoperiod. Plants were acclimated by periodically reducing the ambient humidity starting above 60%. At the end of 30 days, the living plants were kept in the climate chamber under normal conditions.

The plants, which became adaptable to normal climatic conditions after the acclimation step, were grown there for 30 days. At the end of this stage, spike formation was observed in the plants.

3.2.4 Statistical Analysis

The experiment was carried out according to a randomized complete plot design with five replications in petri dishes containing ten embryos. Data on callus formation rate (%), callus weight (g), callus diameter (cm), regeneration capacity (%) and culture effect (%) 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 varieties (Düzgüneş et al. 1987, Saha et al, 2017).

4. RESULTS

4.1 Callus Weight

For mature embryos of two different wheat species (*T. monococcum* subsp. *monococcum* (IZA) and *T. aestivum* L. (Tosunbey)), callus forming media were prepared by applying five different boron concentrations (0, 6.2, 12.4, 24.6 and 37.2 mg/L) and four different concentrations (0, 1, 2 and 4 mg/L) of two different hormone types (2,4-D and Dicamba) to the basic media containing 4.4 g/L MS, 30 g/L sucrose and 8 g/L agar. Mature embryos were placed in the prepared media with the scutellum up and kept in the air conditioning room for 28 days in the dark. Einkorn embryos were transferred into callus media started to form callus within two-three days, bread wheat embryos within six-seven days. ANOVA results with the data obtained by weighing the calli weights formed after 28 days were shown in Table 4.1-4.3.

Comparisons based on different hormone concentrations were evaluated separately. As seen in Table 4.1, at the level of 1 mg/L hormone; in terms of callus weight, the difference between wheat species, boron stress, wheat x boron and boron x hormone was statistically significant at 1% level. There was no significant difference in terms of hormone type, wheat x hormone and wheat x boron x hormone.

Table 4.1. Variance analysis results of different boron stresses and hormone types on callus weights of wheat mature embryos at a hormone concentration level of 1 mg/L.

Source	Sum of Squares	Df	Mean Square	F	Sig.
Wheat Species	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
Wheat X Boron	3.736	4	0.934	5.894	0.000
Wheat X Hormone	0.164	1	0.164	1.040	0.310
Boron X Hormone	3.734	4	0.933	5891	0.000
Wheat X Boron X Hormone	0.549	4	0.137	0.867	0.487
Error	12.676	80	0.158		
Total	42.848	99			

As seen in Table 4.2, at the level of 2 mg/L hormone; the difference between wheat species, boron stress, hormone type, wheat x hormone and boron x hormone in terms of callus weight was statistically significant at 1% level. There was no significant difference in wheat x boron and wheat x boron x hormone.

Table 4.2. Variance analysis of different boron stresses and hormone types on callus weights of wheat mature embryos at a hormone concentration level of 2 mg/L.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	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
Wheat X Boron	1.310	4	0.328	1.590	0.185
Wheat X Hormone	2.283	1	2.283	11.080	0.001
Boron X Hormone	4.796	4	1199	5818	0.000
Wheat X Boron X Hormone	1.780	4	0.445	2.160	0.081
Error	16.485	80	0.206		
Total	46.161	99			

As seen in Table 4.3, at 4 mg/L hormone level; the difference between boron stress, hormone type, wheat x boron and wheat x hormone in terms of callus weight was statistically significant at 1% level. The difference between wheat species was found to be significant at the level of 5%. There was no significant difference in boron x hormone and wheat x boron x hormone.

Table 4.3. Variance analysis results of different boron stresses and hormone types on callus weights of wheat mature embryos at a hormone concentration level of 4 mg/L.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	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
Wheat X Boron	3.382	4	0.846	3.896	0.006
Wheat X Hormone	11.868	1	11.868	54.683	0.000
Boron X Hormone	0.776	4	0.194	0.894	0.471
Wheat X Boron X Hormone	0.917	4	0.229	1.057	0.383
Error	17.363	80	0.217		
Total	61.802	99			

Multiple comparisons test method (Duncan) results to determine the difference between wheat species, boron stress and hormone types were shown in Table 4.4. The results of wheat species under boron stress are shown in Figure 4.1 and 4.2. Images of calli formed at the end of embryo culture are shown in Figure 4.3.

As seen in Table 4.4, the average callus weight in einkorn was found to be between 1.99 g and 3.54 g at 2.4-D. In Dicamba, the average callus weight ranged from 2.02 g to 3.71 g. The highest callus weight was 3.71 in the media containing 1 mg/L Dicamba under 6.2 mg/L Boron stress. The lowest callus weight was 1.99 g in the media containing 4 mg/L 2.4-D under 37.2 mg/L Boron stress.

The average callus weight in bread wheat varied between 1.20 g and 2.57 g at 2.4-D. The average callus weight in Dicamba was between 1.68 g and 3.46 g. The highest callus weight was seen in the media containing 4 mg/L Dicamba under the stress of 24.6 mg/L Boron. The lowest callus weight was found in an environment containing 4 mg/L 2, 4-D under the stress of 37.2 mg/L Boron.

In comparison of the species, it was observed that einkorn wheat constitutes 3.71 g and bread wheat 3.46 g in terms of average callus weight. The lowest values were 1.99 g of einkorn wheat and 1.20 g of bread wheat.

When 2, 4-D and Dicamba concentrations were examined under different boron stresses in terms of callus weight; It is seen that the averages consist of 23 different groups (Table 4.4). When comparing two different types of wheat, the responses of einkorn wheat to different plant growth regulators were more successful under boron toxicity than bread wheat. According to the data obtained, einkorn wheat formed more calli than bread wheat.

Table 4.4. Multiple comparisons test results to determine the difference between wheat species, boron stress and hormone types according to callus weight.

Wheat Species	Boron Concentrations (mg/L)	Hormone Types	Hormone Concentrations (mg/L)	Callus Weight (g)*
Einkorn (*T. monococcum* subsp. m*onococcum*) (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
	6.2	2, 4-D	Control (0)	-
			1	3.54 ± 0.35 abc
			2	3.03 ± 0.55 cdefghi
			4	2.88 ± 0.60 efghijkl
		Dicamba	Control (0)	-
			1	3.71 ± 0.13 a
			2	3.54 ± 0.32 abcd
			4	3.64 ± 0.16 ab
	12.4	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
	24.6	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
	37.2	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

*Different letters in the same column indicate significantly differences at $p < 0.05$.

Table 4.4. Multiple comparisons test results to determine the difference between wheat species, boron stress and hormone types according to callus weight (**Table 4.4. Cont'd**).

Wheat Species	Boron Concentrations (mg/L)	Hormone Types	Hormone Concentrations (mg/L)	Callus Weight (g)*
Bread Wheat (*T. aestivum* L.) (Tosunbey)	0	2,4-D	Control (0)	-
			1	1.97 ± 0.44 nopqrst
			2	2.14 ± 1.02 mnopqrst
			4	1.77 ± 0.35 pqrstuv
		Dicamba	Control (0)	-
			1	2.60 ± 0.40 ghijklmn
			2	3.24 ± 0.37 abcdef
			4	3.04 ± 0.59 bcdefgh
	6.2	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 in the same column indicate significantly differences at $p < 0.05$.

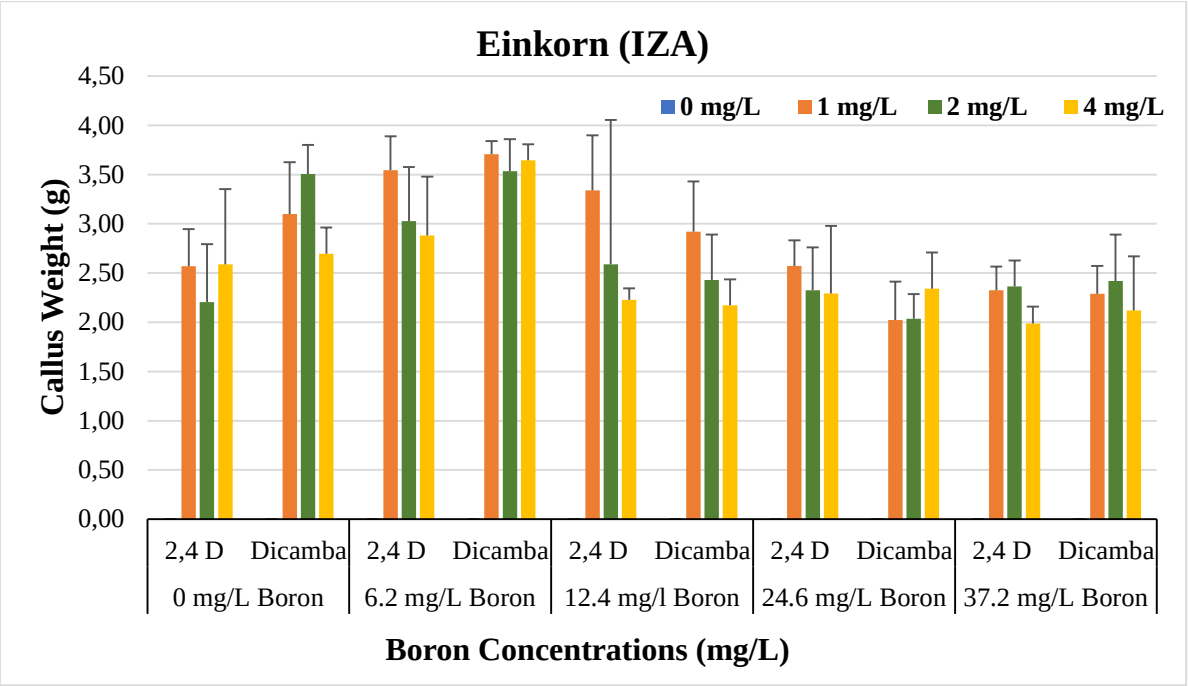

Figure 4.1. Callus weight results on einkorn wheat of different constants of two different hormone types under boron stress.

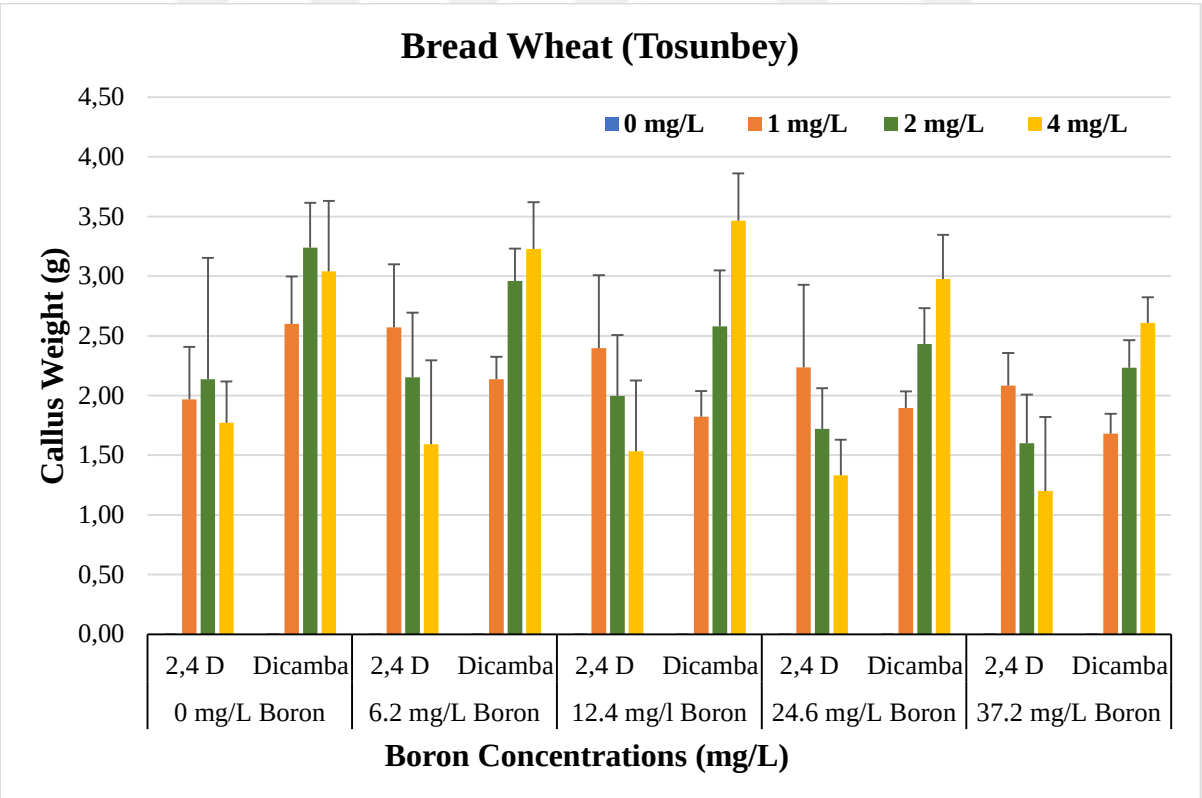

Figure 4.2. Callus weight results on bread wheat of different constants of two different hormone types under boron stress.

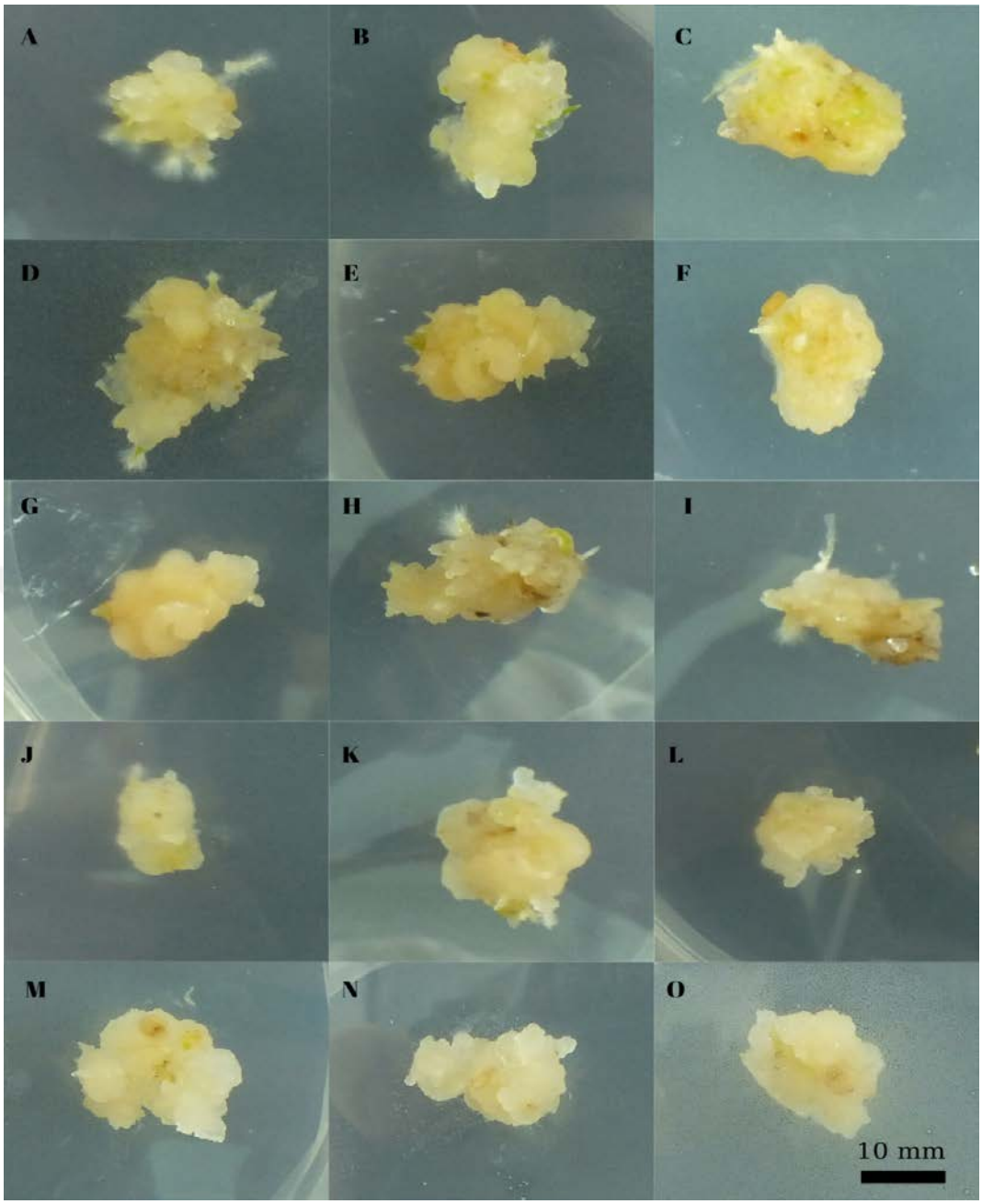

Figure 4.3. Einkorn wheat weighed before transfer to regeneration media. **A:** 1 mg/L Dicamba, **B:** 2 mg/L Dicamba, **C:** 4 mg/L Dicamba (A, B and C under 0 mg/L Boron). **D**: 1 mg/L Dicamba, **E:** 2 mg/L Dicamba, **F:** 4 mg/L Dicamba (D, E and F under 6.2 mg/L Boron). **G:** 1 mg/L Dicamba, **H:** 2 mg/L Dicamba, **I:** 4 mg/L Dicamba (G, H and I 12.4 mg/L under 0 mg/L Boron). **J:** 1 mg/L Dicamba, **K**: 2 mg/L Dicamba, **L:** 4 mg/L Dicamba (J, K and L under 24.6 mg/L Boron). **M:** 1 mg/L Dicamba, **N:** 2 mg/L Dicamba, **O:** 4 mg/L Dicamba (M, N and O under 37.2 mg/L Boron)

4.2 Callus Diameter (cm)

For mature embryos of two different wheat species, five different boron concentrations and four different concentrations of two different hormone types were applied to callus media. Mature embryos were placed in the prepared environments as the scutellum up and kept in the air conditioning room for 28 days in the dark. ANOVA with the data obtained by measuring the callus diameters formed at the end of 28 days with a ruler are shown in Table 4.5-4.7.

As seen in Table 4. 5, at the level of 1 mg/L hormone; the difference between Hormone types in terms of callus diameter was statistically significant at 1% level. A 5% significant difference was found between Wheat species and wheat x boron stress x hormone. There was no significant difference in boron stress, wheat x boron, wheat x hormone and boron x hormone.

Table 4.5. Variance analysis results of different boron stresses and hormone types on callus diameter of wheat mature embryos at a hormone concentration level of 1 mg/L.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	0.372	1	0.372	4.587	0.032
Boron Stress	0.693	4	0.173	2.134	0.075
Hormone Type	3.170	1	3.170	39.032	0.000
Wheat X Boron	0.457	4	0.114	1.407	0.229
Wheat X Hormone	0.210	1	0.210	2.589	0.108
Boron X Hormone	0.617	4	0.154	1901	0.108
Wheat X Boron X Hormone	0.831	4	0.208	2.558	0.037
Error	79.583	980	0.081		
Total	85.934	999			

As seen in Table 4.6, at the level of 2 mg/L hormone; in terms of callus diameter, the difference between wheat species, hormone types, wheat x boron stress and wheat x hormone was statistically significant at 1% level. There was no significant difference in boron stress, boron x hormone and wheat x boron x hormone.

Table 4.6. Variance analysis results of different boron stresses and hormone varieties on callus diameter of wheat mature embryos at a hormone concentration level of 2 mg/L.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	1.267	1	1.267	17.065	0.000
Boron Stress	0.244	4	0.061	0.821	0.512
Hormone Type	8.987	1	8.987	121.014	0.000
Wheat X Boron	1.512	4	0.378	5.090	0.000
Wheat X Hormone	0.605	1	0.605	8.149	0.004
Boron X Hormone	0.393	4	0.098	1323	0.260
Wheat X Boron X Hormone	0.331	4	0.083	1.115	0.348
Error	72.779	980	0.074		
Total	86.119	999			

As seen in Table 4. 7, at 4 mg/L hormone level; in terms of callus diameter, the difference between wheat species, hormone, wheat x hormones and wheat x boron x hormones was statistically significant at 1% level. There was no significant difference between boron stress, wheat x boron and boron x hormones.

Table 4.7. Variance analysis results of different boron stresses and hormone types on callus diameter of wheat mature embryos at a hormone concentration level of 4 mg/L.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	1.011	1	1.011	12.380	0.000
Boron Stress	0.214	4	0.054	0.656	0.623
Hormone Type	9.565	1	9.565	117.098	0.000
Wheat X Boron	0.766	4	0.192	2.345	0.053
Wheat X Hormone	4.303	1	4.303	52.684	0.000
Boron X Hormone	0.720	4	0.180	2204	0.067
Wheat X Boron X Hormone	1138	4	0.285	3.483	0.008
Error	80.048	980	0.082		
Total	97.766	999			

Multiple comparisons test method (Duncan) results for determining the difference between Wheat species, Boron Stress and Hormone types are shown in table 4.8. The results of wheat species under boron stress are shown in Figure 4.4

and 4.5. Images of calli formed at the end of embryo culture are shown in Figure 4.6.

As seen in Table 4.8, the average callus diameter in einkorn was found to be between 0.79 cm and 0.95 cm in 2.4-D. In Dicamba, the average callus diameter ranged from 0.87 cm to 1.10 cm. The highest callus diameter was 1.10 cm in the media containing 1 mg/L Dicamba under 12.4 mg/L Boron stress. The lowest callus diameter was 0.79 cm in an environment containing 1 mg/L 2.4-D under 12.4 mg/L Boron stress.

The average callus diameter in bread wheat varied between 0.78 cm and 1.01 cm at 2.4-D. The average callus diameter in Dicamba was between 0.98 cm and 1.22 cm. The highest callus diameter was 1.22 cm in the media containing 4 mg/L Dicamba under 6.2 mg/L boron stress. The lowest callus diameter was 0.78 cm in the media containing 2 mg/L 2,4-D under 12.4 mg/L boron stress.

In the comparison between the species, in terms of the highest average callus diameter, einkorn wheat was 1.10 cm and bread wheat were 1.22 cm. The lowest values were 0.79 cm of einkorn wheat and 0.78 cm of bread wheat.

When 2, 4-D and Dicamba concentrations were examined under different boron stresses in terms of callus diameter; it is seen that the averages consist of 22 different groups (Table 4.8).

Table 4.8. Multiple comparisons test results to determine the difference between wheat species, boron stress, and hormone types according to callus diameter.

Wheat Species	Boron Concentrations (mg/L)	Hormone Types	Hormone Concentrations (mg/L)	Callus Diameter (cm)*
Einkorn (*T. monococcum* subsp. m*onococcum*) (IZA)	0	2,4-D	Control (0)	-
			1	0.93 ± 0.26 jklmnop
			2	0.84 ± 0.34 opqrstu
			4	0.92 ± 0.32 klmnopqr
		Dicamba	Control (0)	-
			1	1.01 ± 0.39 fghijkl
			2	1.00 ± 0.32 ghijklm
			4	0.87 ± 0.27 nopqrstu
	6.2	2,4-D	Control (0)	
			1	0.89 ± 0.32 lmnopqrstu
			2	0.80 ± 0.30 stu
			4	0.92 ± 0.31 jklmnopq
		Dicamba	Control (0)	
			1	0.94 ± 0.18 jklmnop
			2	0.88 ± 0.18 mnopqrstu
			4	0.89 ± 0.19 lmnopqrstu
	12.4	2,4-D	Control (0)	
			1	0.79 ± 0.28 u
			2	0.87 ± 0.27 nopqrstu
			4	0.91 ± 0.33 klmnopqrst
		Dicamba	Control (0)	
			1	1.10 ± 0.31 bcdefgh
			2	1.01 ± 0.28 fghijk
			4	1.02 ± 0.31 fghijk
	24.6	2,4-D	Control (0)	-
			1	0.86 ± 0.35 opqrstu
			2	0.85 ± 0.31 opqrstu
			4	0.92 ± 0.28 jklmnop
		Dicamba	Control (0)	-
			1	1.02 ± 0.25 fghijk
			2	0.98 ± 0.22 hijklmn
			4	1.02 ± 0.22 fghijk
	37.2	2,4-D	Control (0)	-
			1	0.95 ± 0.27 ijklmno
			2	0.88 ± 0.29 mnopqrstu
			4	0.82 ± 0.29 pqrstu
		Dicamba	Control (0)	-
			1	1.06 ± 0.26 efghi
			2	1.08 ± 0.27 defgh
			4	1.01 ± 0.30 fghijk

*Different letters in the same column indicate significantly differences at $p < 0.05$.

Table 4.8. Multiple comparisons test results to determine the difference between wheat species, boron stress and hormone types according to callus diameter (**Table 4.8. Cont'd**).

Wheat Species	Boron Concentrations (mg/L)	Hormone Types	Hormone Concentrations (mg/L)	Callus Diameter (cm)*
Bread Wheat (*T.aestivum* L.) (Tosunbey)	0	2,4-D	Control (0)	-
			1	0.98 ± 0.36 hijklmn
			2	0.87 ± 0.34 nopqrstu
			4	0.88 ± 0.29 mnopqrstu
		Dicamba	Control (0)	-
			1	1.11 ± 0.27 abcdefg
			2	1.17 ± 0.28 abcd
			4	1.20 ± 0.26 ab
	6.2	2,4-D	Control (0)	-
			1	0.95 ± 0.30 ijklmno
			2	0.92 ± 0.29 klmnopqrs
			4	0.80 ± 0.33 ru
		Dicamba	Control (0)	-
			1	1.04 ± 0.28 efghij
			2	1.15 ± 0.25 abcde
			4	1.22 ± 0.27 a
	12.4	2,4-D	Control (0)	-
			1	0.88 ± 0.26 mnopqrstu
			2	0.78 ± 0.25 u
			4	0.80 ± 0.30 qrstu
		Dicamba	Control (0)	-
			1	0.98 ± 0.29 hijklmn
			2	1.09 ± 0.24 cdefgh
			4	1.19 ± 0.37 abc
	24.6	2,4-D	Control (0)	
			1	1.01 ± 0.26 fghijk
			2	0.94 ± 0.27 jklmnop
			4	0.87 ± 0.30 nopqrstu
		Dicamba	Control (0)	- -
			1	0.99 ± 0.25 hijklmn
			2	1.06 ± 0.25 defghi
			4	1.06 ± 0.25 efghi
	37.2	2,4-D	Control (0)	-
			1	0.93 ± 0.27 jklmnop
			2	0.86 ± 0.27 opqrstu
			4	0.79 ± 0.28 tu
		Dicamba	Control (0)	-
			1	1.04 ± 0.24 efghij
			2	1.07 ± 0.19 defgh
			4	1.12 ± 0.20 abcdef

*Different letters in the same column indicate significantly differences at $p < 0.05$.

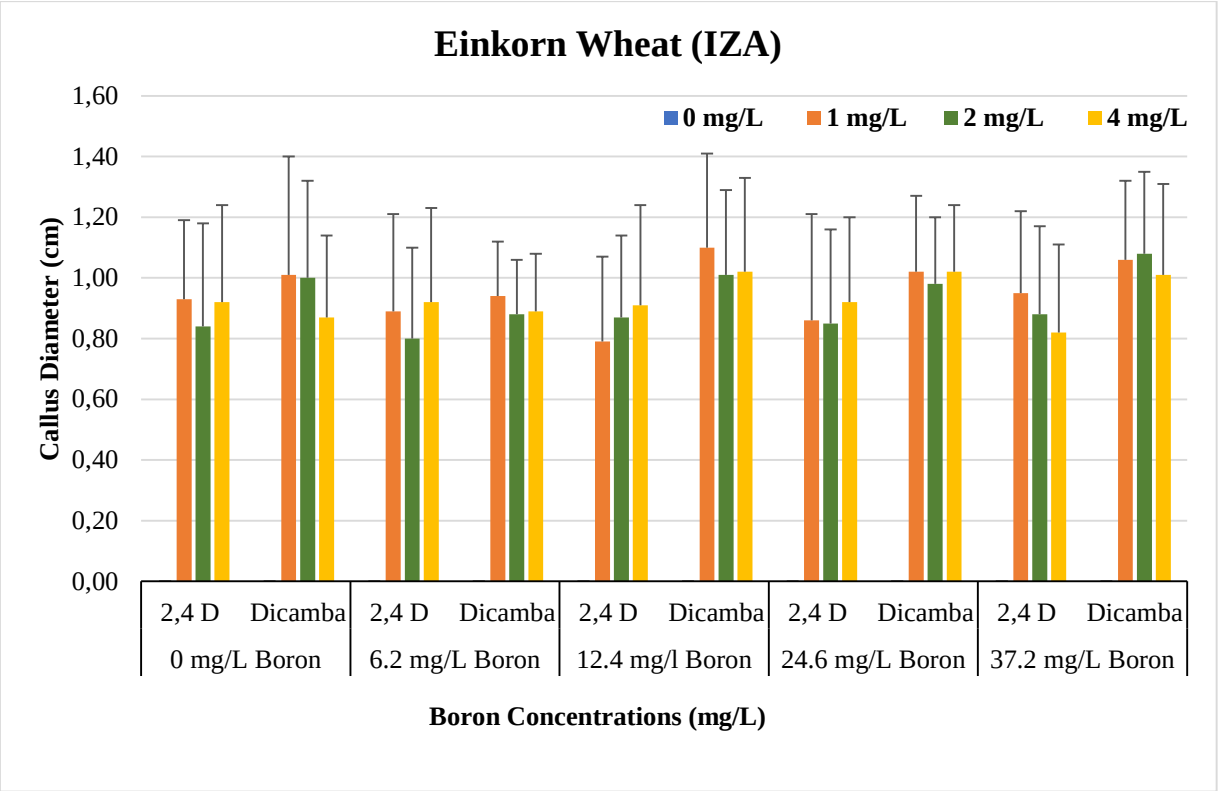

Figure 4.4. Callus diameter results on einkorn wheat of different constants of two different hormone types under boron stress.

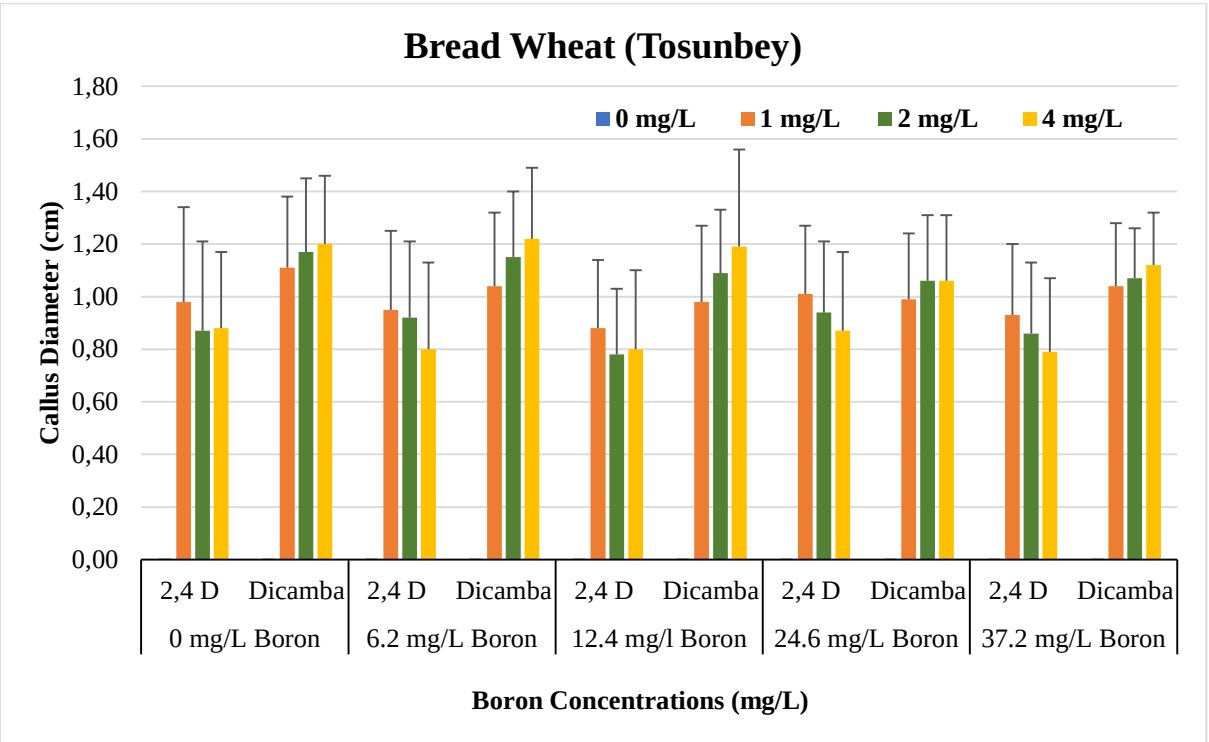

Figure 4.5. Callus diameter results on bread wheat of different constants of two different hormone types under boron stress

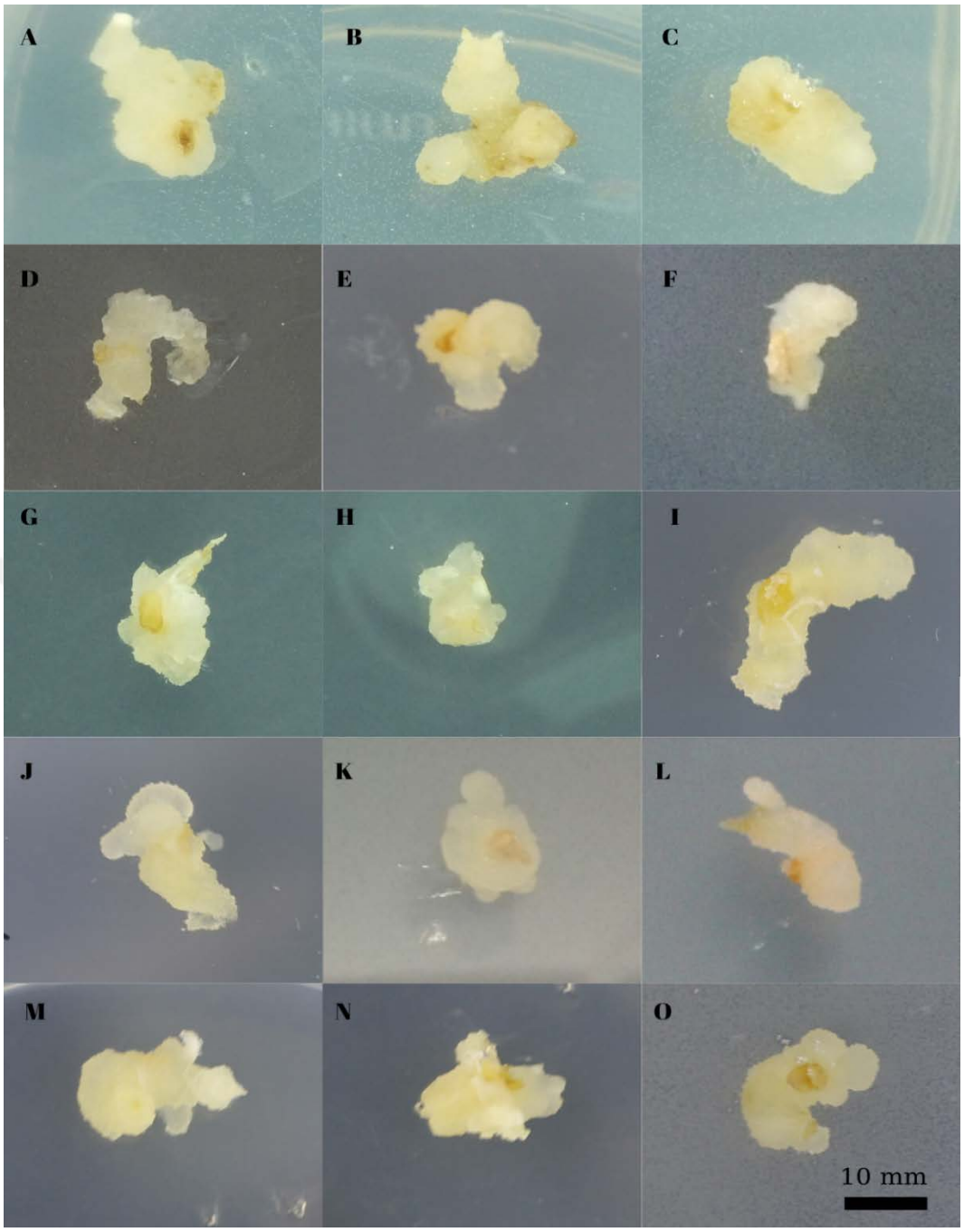

Figure 4.6. Bread wheat measured before transfer to regeneration media. **A:** 1 mg/L 2,4-D, **B:** 2 mg/L 2,4-D, **C:**4 mg/L 2,4-D (A, B and C under 0 mg/L Boron). **D:** 1 mg/L 2,4-D, **E:** 2 mg/L 2,4-D, **F:** 4 mg/L 2,4-D (D, E and F under 6.2 mg/L Boron). **G:** 1 mg/L 2,4-D, **H:** 2 mg/L 2,4-D, **I:** 4 mg/L 2,4-D (G, H and I 12.4 mg/L under 0 mg/L Boron). **J:** 1 mg/L 2,4-D, **K:** 2 mg/L 2,4-D, **L:** 4 mg/L 2,4-D (J, K and L under 24.6 mg/L Boron). **M:** 1 mg/L 2,4-D, **N:** 2 mg/L 2,4-D, **O:** 4 mg/L Dicamba (M, N and O under 37.2 mg/L Boron)

4.3 Regeneration Capacity (%)

Mature embryos of two different wheat species were allowed to form callus for 28 days under five different boron stresses, in the media containing four different concentrations of 2, 4-D and Dicamba hormones. The calli that were formed were taken to the regeneration environment containing three different doses of BAP hormone (0, 0.5, and 2 mg/L) on the 28^{th} day, and kept in the air-conditioning room for 16 days in a bright 8 hours in the dark, 23 ± 1 temperature and 55% humidity for 28 days.

As seen in Table 4.9, at the level of 0 mg/L hormone; in terms of regeneration capacity, a statistically significant difference of 1% was observed between boron stress and wheat x boron. There was no significant difference between wheat species.

Table 4.9. Variance analysis results of different boron stresses and wheat species on regeneration capacity of wheat mature embryos at a hormone concentration level of 0 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	160	1	160	2	0.221
Boron Stress	34.964	4	8.741	83	0
Wheat X Boron	2.948	4	737	7	0
Error	9.516	90	105,728		
Total	47.588	99			

As seen in Table 4.10, at the level of 0.5 mg/L hormone; in terms of regeneration capacity, Boron Stress difference was statistically significant at 1% level. A 5% significant difference was found between wheat x boron. There was no significant difference in Wheat species.

Table 4.10. Variance analysis results of different boron stresses and wheat species on regeneration capacity of wheat mature embryos at a hormone concentration level of 0.5 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	11	1	11	0	0.734
Boron Stress	17.695	4	4.424	46	0
Wheat X Boron	1.044	4	261	3	0.034
Error	8.600	90	95.553		
Total	27.351	99			

As seen in Table 4.11, at the level of 2 mg/L hormone; in terms of regeneration capacity, boron stress was found statistically significant at 1% level. There was no significant difference in wheat species and wheat x boron.

Table 4.11. Variance analysis results of different boron stresses and wheat species on regeneration capacity of wheat mature embryos at a hormone concentration level of 2 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	545	1	545	4	0.06
Boron Stress	20.345	4	5.086	34	0
Wheat X Boron	507	4	127	1	0.502
Error	13.542	90	150.472		
Total	34.939	99			

Multiple comparisons test method (Duncan) results are shown in table 4.12 to determine the difference between wheat species, boron stress and hormone concentrations. The results of wheat species under boron stress are shown in figure 4.7. Images of calli formed at the end of embryo culture are shown in the Figure in 4.8-4.10.

As seen in Table 4.12, einkorn regeneration capacity was found to be between 6 and 71.33%. The highest regeneration capacity was 71.33% in media containing 0 mg/L BAP under 6.2 mg/L Boron stress. The lowest regeneration capacity was 6% in an environment containing 2 mg/L BAP under 37.2 mg/L Boron stress.

Regeneration capacity in bread wheat varied between 7.33% and 65.33%. The highest regeneration capacity was 65.33% in media containing 0 mg/L BAP under 6.2 mg/L Boron stress. The lowest regeneration capacity was 7.33% in an environment containing 0.5 mg/L BAP under 24.6 mg/L Boron stress.

In the comparison between species, it was observed that einkorn wheat constituted 71.33% and bread wheat 65.33% in terms of regeneration capacity. The lowest values were 6% of einkorn wheat and 7.33% of bread wheat.

When 2, 4-D and Dicamba concentrations are examined under different boron stresses in terms of callus diameter; it is seen that the averages consist of 12 different groups (Table 4.12).

Table 4.12. Multiple comparisons test results to determine the difference between wheat species, boron stress and hormone concentrations according to regeneration capacity.

Wheat Species	Boron Concentrations (mg/L)	Hormone Concentrations (mg/L) (BAP)	Regeneration Capacity (%) *
Einkorn (*T. monococcum* subsp. m*onococcum*) (IZA)	0	Control (0)	38.00 ± 9.96 eg
		0.5	30.00 ± 11.86 fh
		2	34.67 ± 12.88 fg
	6.2	Control (0)	71.33 ± 11.78 a
		0.5	48.00 ± 9.84 ce
		2	56.67 ± 12.27 bc
	12.4	Control (0)	31.33 ± 10.45 fh
		0.5	22.00 ± 13.35 hj
		2	28.67 ± 10.91 fi
	24.6	Control (0)	18.00 ± 12.97 ik
		0.5	14.67 ± 10.80 jl
		2	13.33 ± 11.76 jl
	37.2	Control (0)	14.67 ± 12.88 jl
		0.5	8.00 ± 9.32 kl
		2	6.00 ± 7.34 lp
Bread Wheat (T. *aestivum* L.) (Tosunbey)	0	Control (0)	28.67 ± 8.34 fi
		0.5	28.67 ± 9.45 fi
		2	28.00 ± 11.68 gi
	6.2	Control (0)	65.33 ± 10.80 ab
		0.5	46.00 ± 10.16 de
		2	49.33 ± 13.77 cd
	12.4	Control (0)	40.00 ± 7.70 df
		0.5	34.00 ± 6.63 fg
		2	34.00 ± 9.66 fg
	24.6	Control (0)	16.00 ± 15.14 jl
		0.5	7.33 ± 5.84 kl
		2	16.00 ± 11.84 jl
	37.2	Control (0)	20.00 ± 10.89 hj
		0.5	15.33 ± 6.33 jl
		2	22.00 ± 14.76 hj

*Different letters in the same column indicate significantly differences at $p < 0.05$.

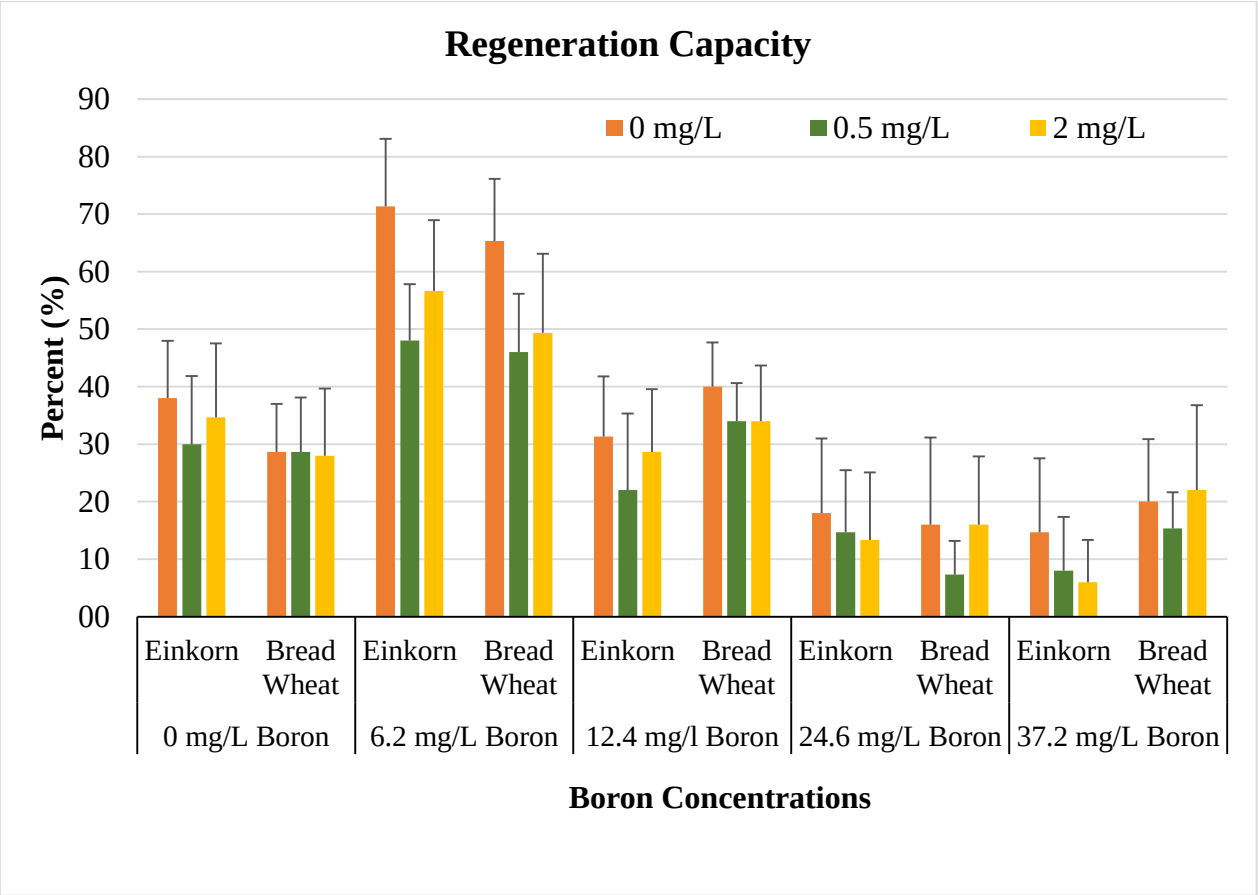

Figure 4.7. Regeneration capacity results on einkorn and bread wheat of different constants of BAP hormone under boron stress.

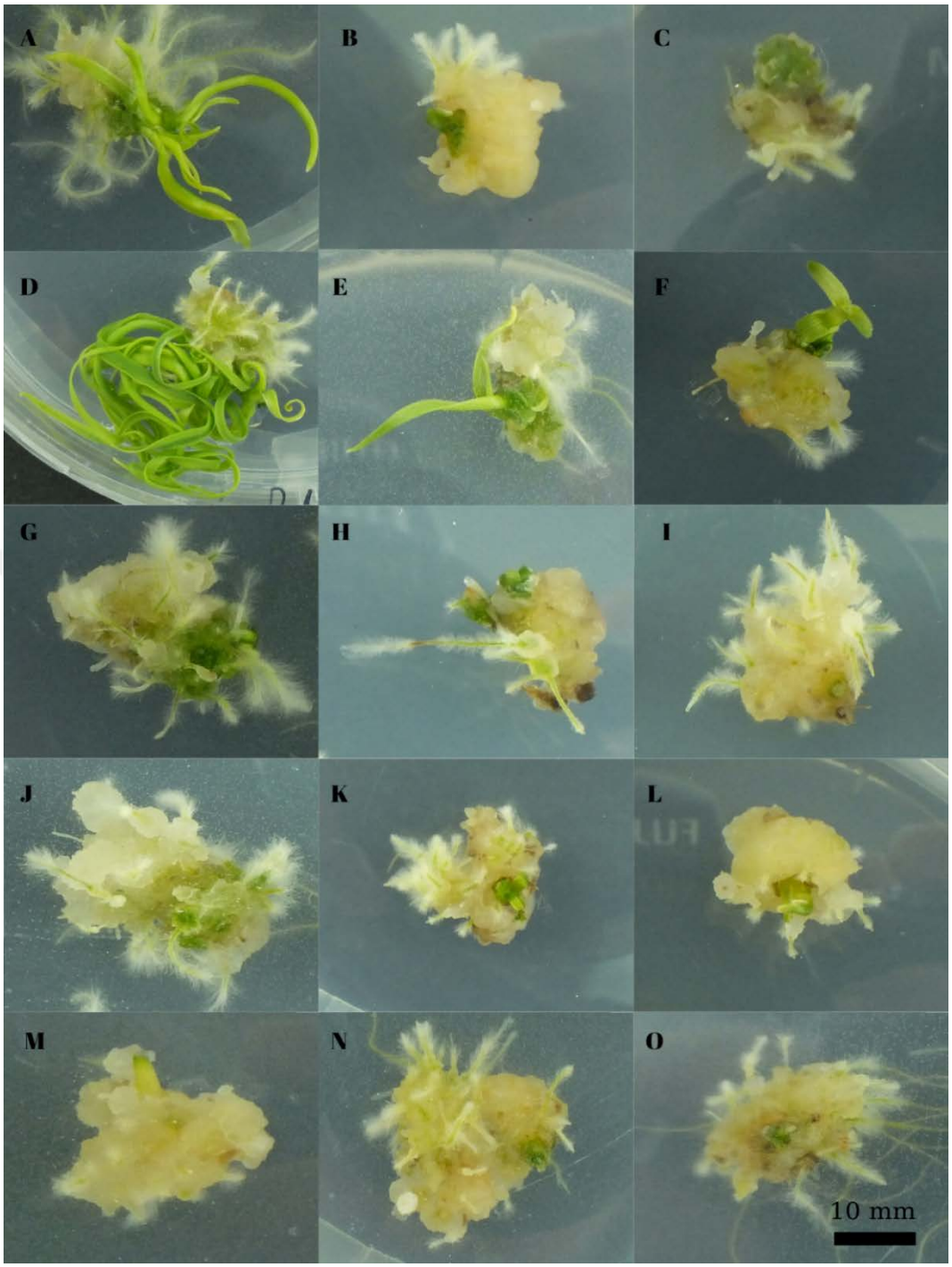

Figure 4.8. Bread wheat mature embryo callus forming regeneration. **A:** 0 mg/L BAP, **B:** 0.5 mg/L BAP, **C:**2 mg/L BAP (A, B and C under 0 mg/L Boron). **D:** 0 mg/L BAP, **E:** 0.5 mg/L BAP, **F**:2 mg/L BAP (D, E and F under 6.2 mg/L Boron). **G:** 0 mg/L BAP, **H:** 0.5 mg/L BAP, **I:** 2 mg/L BAP (G, H and I 12.4 mg/L under 0 mg/L Boron). **J:** 0 mg/L BAP, **K:** 0.5 mg/L BAP, **L:** 2 mg/L BAP (J, K and L under 24.6 mg/L Boron). **M:** 0 mg/L BAP, **N:** 0.5 mg/L BAP, **O:**2 mg/L BAP (M, N and O under 37.2 mg/L Boron)

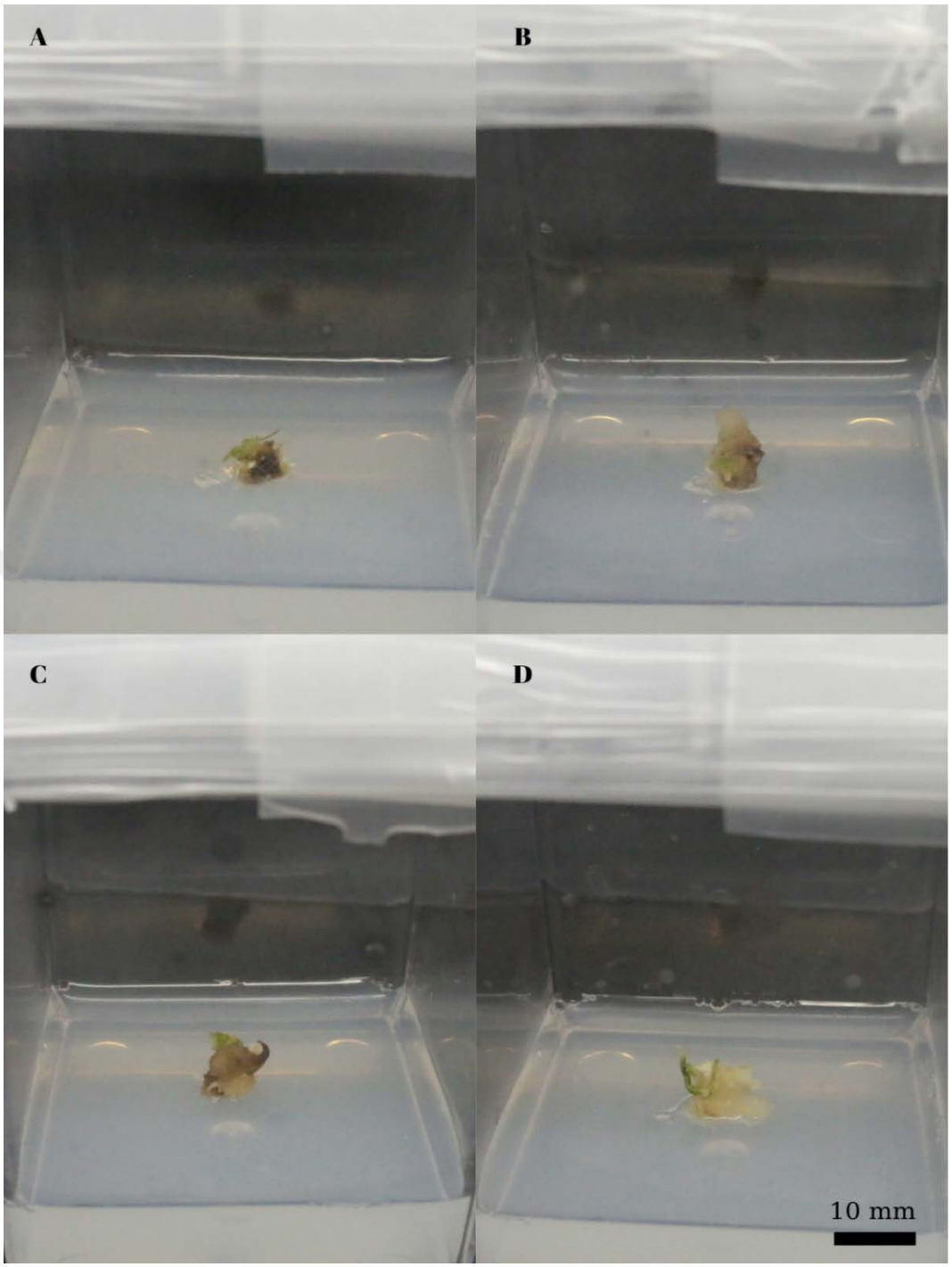

Figure 4.9. Einkorn wheat mature embryo callus forming regeneration. **A:** 0 mg/L BAP under 0 mg/L Boron. **B:** 0 mg/L BAP under 0 mg/L Boron. **C:** 0 mg/L BAP under 0 mg/L Boron. **D:** 0.5 mg/L BAP under 6.2 mg/L Boron.

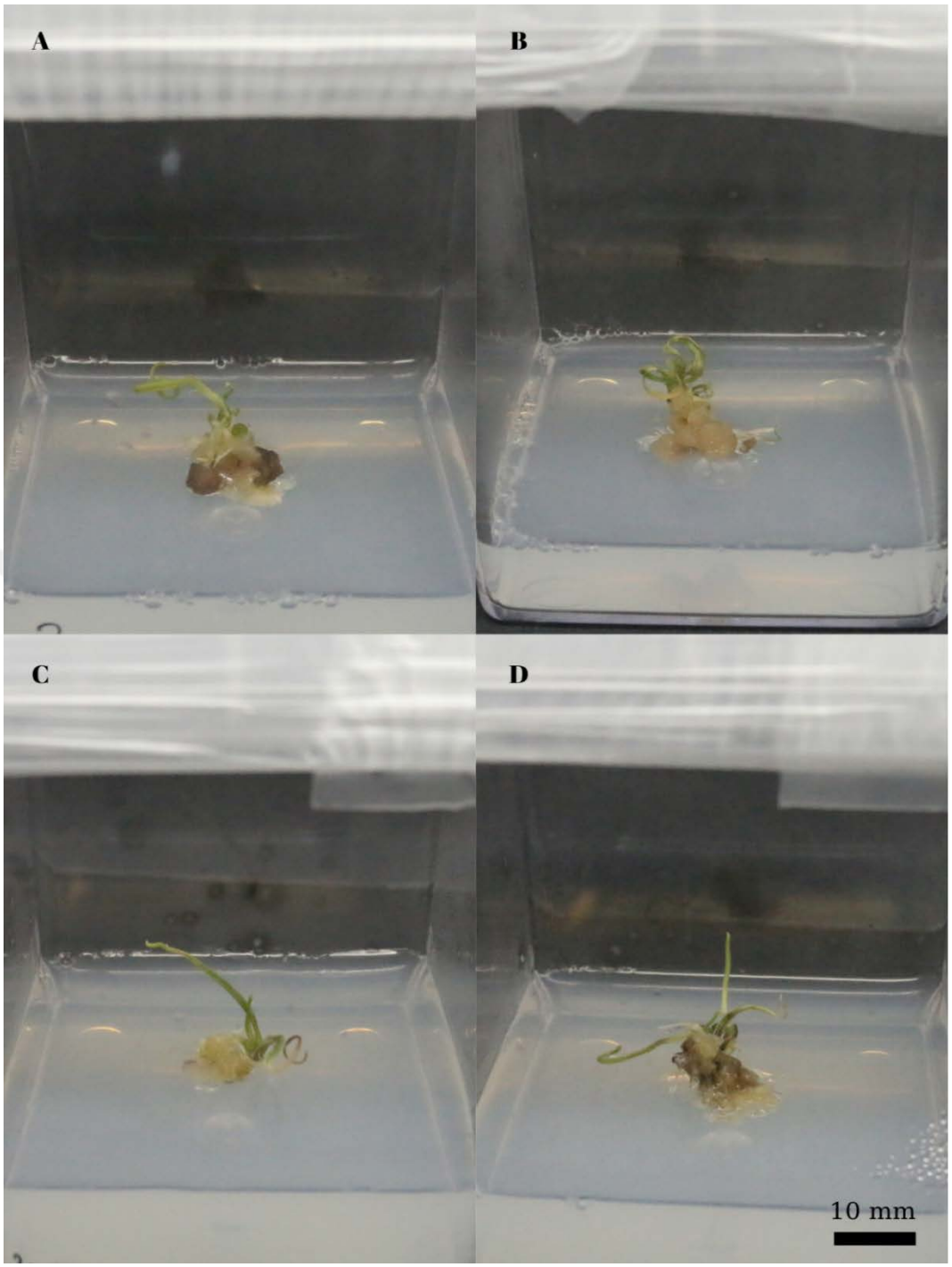

Figure 4.10. Einkorn wheat mature embryos forming regeneration. **A:** 0 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron. **C:** 2 mg/L BAP under 6.2 mg/L Boron. **D:** 2 mg/L BAP under 6.2 mg/L Boron.

4.4 Number of Plantlets (n)

Mature embryos of two different wheat species were allowed to form callus for 28 days under five different Boron stresses in the media containing four different concentrations of 2, 4-D and Dicamba hormones. The calli that were formed were taken to the regeneration environment containing 3 different doses of BAP hormone on the 28th day, and kept in the air-conditioning room for 16 days in a bright 8 hours in the dark, 23 ± 1 temperature and 55% humidity for 28 days. The regenerated shoots reaching 4-5 cm in length at the end of 28 days were transferred to the magenta boxes with the content of the regeneration media to ensure root development and shoot elongation. The transferred samples were kept in 16 hours of light and 8 hours of darkness in 28 days. The results of the variance analysis obtained by counting the number of plants obtained by counting the samples that provide root and shoot growth at the end of this stage are shown in Table 4.13-15.

As seen in Table 4.13, at the level of 0 mg/L hormone; there was a statistically significant difference of 1% between boron stress and wheat x boron in terms of the number of plants. There was no significant difference in wheat species.

Table 4.13. Variance analysis results of different boron stresses and wheat species on plantlets number of wheat mature embryos at a hormone concentration level of 0 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	1.296	1	1.296	0.632	0.427
Boron Stress	108.166	4	27.042	13.197	0.000
Wheat X Boron	114.374	4	28.594	13.955	0.000
Error	2028.560	990	2049		
Total	2252.396	999			

As seen in Table 4.14, at the level of 0 mg/L hormone; in terms of number of plants, Boron Stress was statistically significant at 1% level. The difference between wheat x boron was 5%. There was no significant difference in wheat species.

Table 4.14. Variance analysis results of different boron stresses and wheat species on plantlets number of wheat mature embryos at a hormone concentration level of 0.5 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	0.049	1	0.049	0.025	0.874
Boron Stress	127.304	4	31.826	16.439	0.000
Wheat X Boron	23.836	4	5.959	3.078	0.016
Error	1916.690	990	1936		
Total	2067.879	999			

As seen in Table 4.15, at the level of 0 mg/L hormone; in terms of number of plants, boron stress was statistically significant at 1% level. There was no significant difference in wheat species and wheat x boron.

Table 4.15. Variance analysis results of different boron stresses and wheat species on plantlets number of wheat mature embryos at a hormone concentration level of 2 mg/L BAP.

Source	Sum of Squares	df	Mean Square	F	Sig.
Wheat Species	0.529	1	0.529	0.308	0.579
Boron Stress	194.586	4	48.647	28.283	0.000
Wheat X Boron	2.586	4	0.647	0.376	0.826
Error	1702.770	990	1720		
Total	1900.471	999			

Multiple comparisons test method (Duncan) results are shown in table 4.16 to determine the difference between wheat species, boron stress and hormone concentrations. The results of wheat species under boron stress are shown in figure 4.11. Images of the plantlets formed at the end of the embryo culture are shown in the figure in 4.12-4.13.

As seen in Table 4.16, the average number of plantlets in einkorn was found to be between 1.33 and 3.08. The highest number of plantlets was 3.08 in media containing 0 mg/L BAP under 12.4 mg/L Boron stress. The lowest number of plantlets was 0.13 in an environment containing 2 mg/L BAP under 37.2 mg/L Boron stress.

The average number of plantlets in bread wheat varies between 1.29 and 3.71. The highest number of plantlets was 3.71 in an environment containing 0.5 mg/L BAP under 6.2 mg/L Boron stress. The lowest number of plantlets was 1.29 in an environment containing 0.5 mg/L BAP under the stress of 24.6 mg/L Boron.

In the comparison between the species, it was observed that einkorn wheat was 3.08 and Bread wheat was 3.71 in terms of the highest number of plantlets. The lowest values were 1.33 of einkorn wheat and 1.29 of bread wheat.

When 2,4-D and Dicamba concentrations are examined under different boron stresses in terms of callus diameter; it is seen that the averages consist of 11 different groups (Table 4.16).

Table 4.16. Multiple comparisons test results to determine the difference between wheat species, boron stress and hormone concentrations according to plantlets number.

Wheat Species	Boron Concentrations (mg/L)	Hormone Concentrations (mg/L) (BAP)	Regeneration Capacity (%)
Einkorn (*T. monococcum* subsp. m*onococcum*) (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 (*T.aestivum*) (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 in the same column indicate significantly differences at $p < 0.05$.

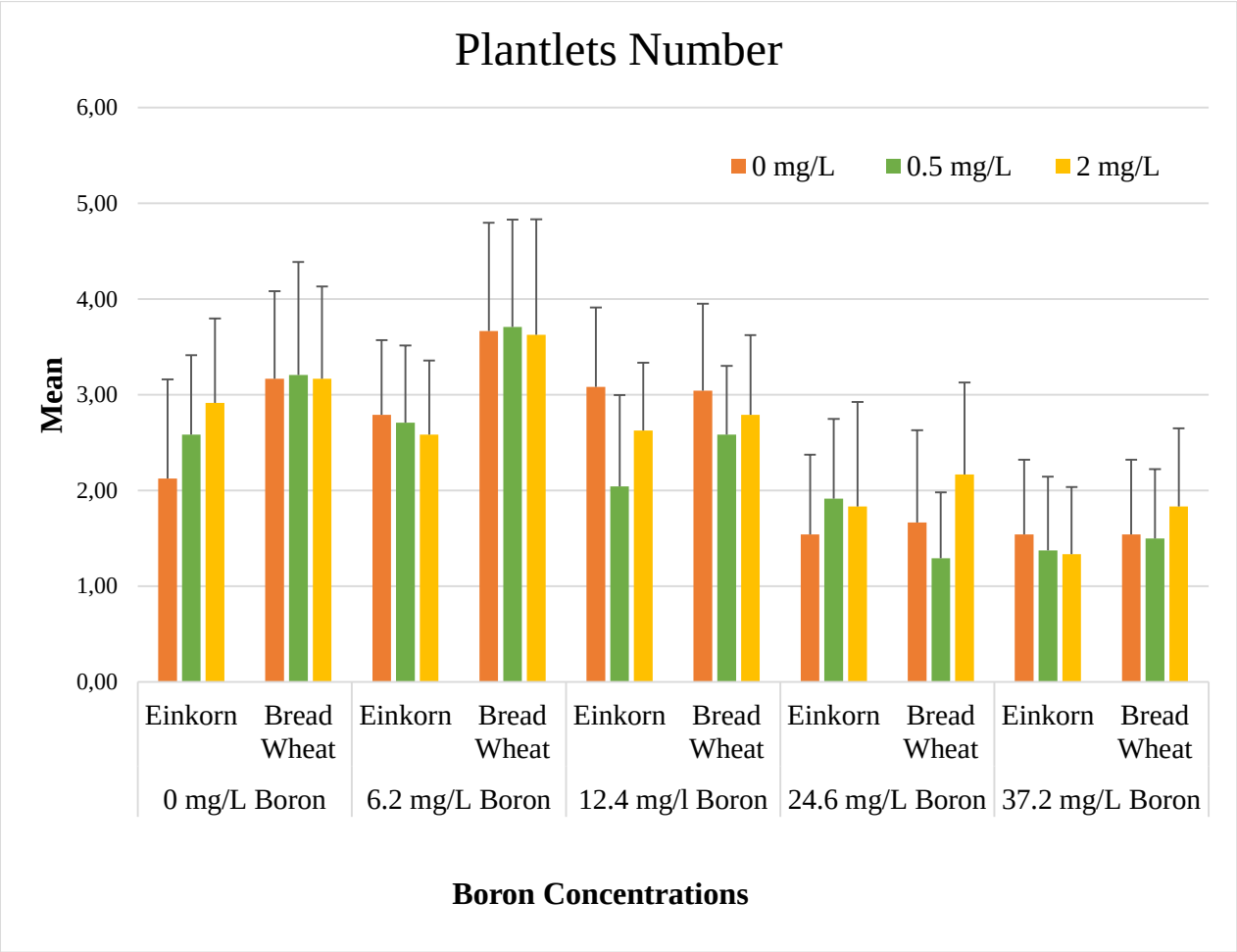

Figure 4.11. Plantlets number results on einkorn and bread wheat of different constants of BAP hormone under boron stress.

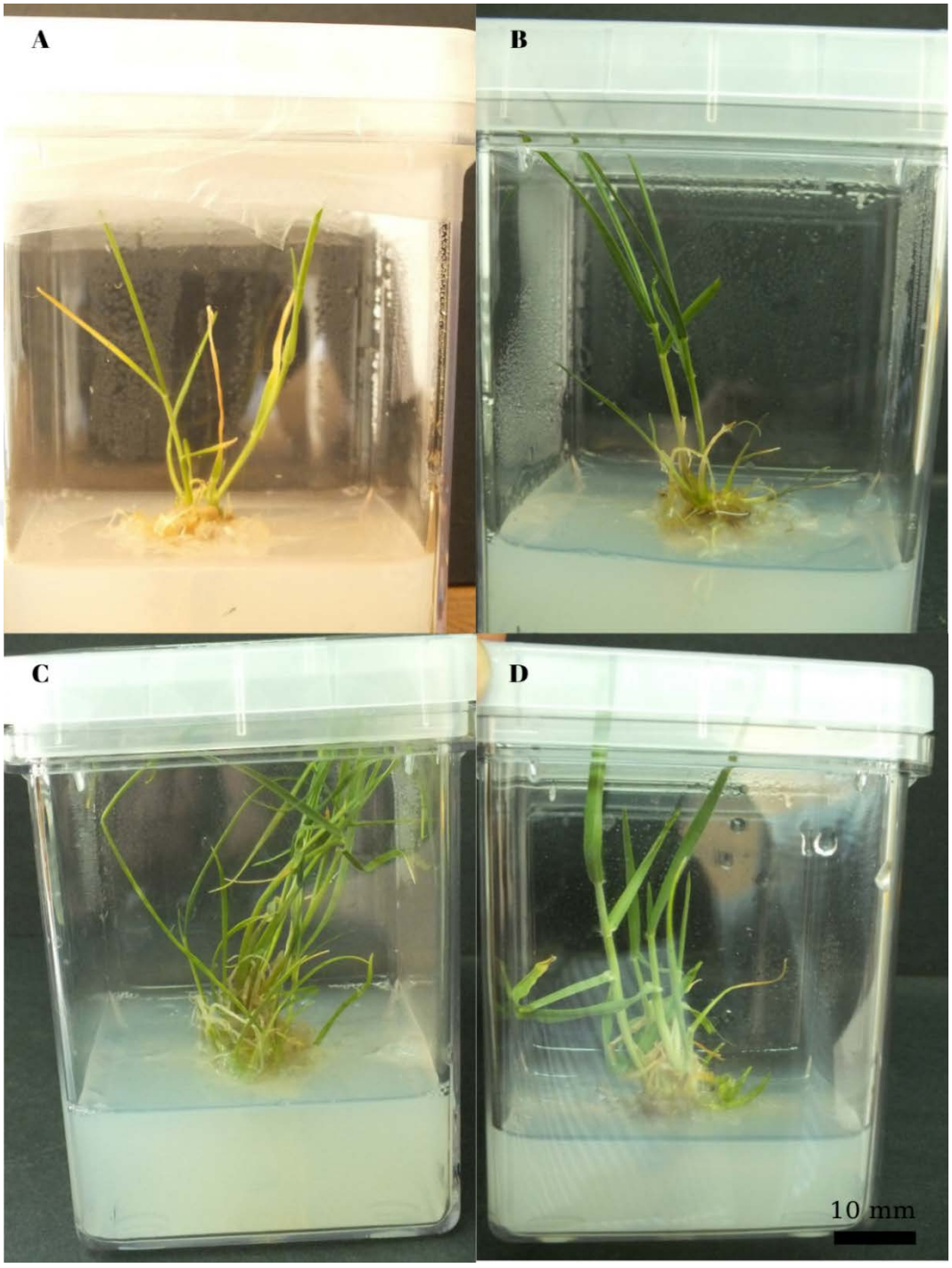

Figure 4.12. Einkorn wheat mature embryos forming plantlets. **A:** 0.5 mg/L BAP under 12.4 mg/L Boron. **B:** 0 mg/L BAP under 6.2 mg/L Boron. **C:** 2 mg/L BAP under 12.4 mg/L Boron. **D:** 2 mg/L BAP under 6.2 mg/L Boron.

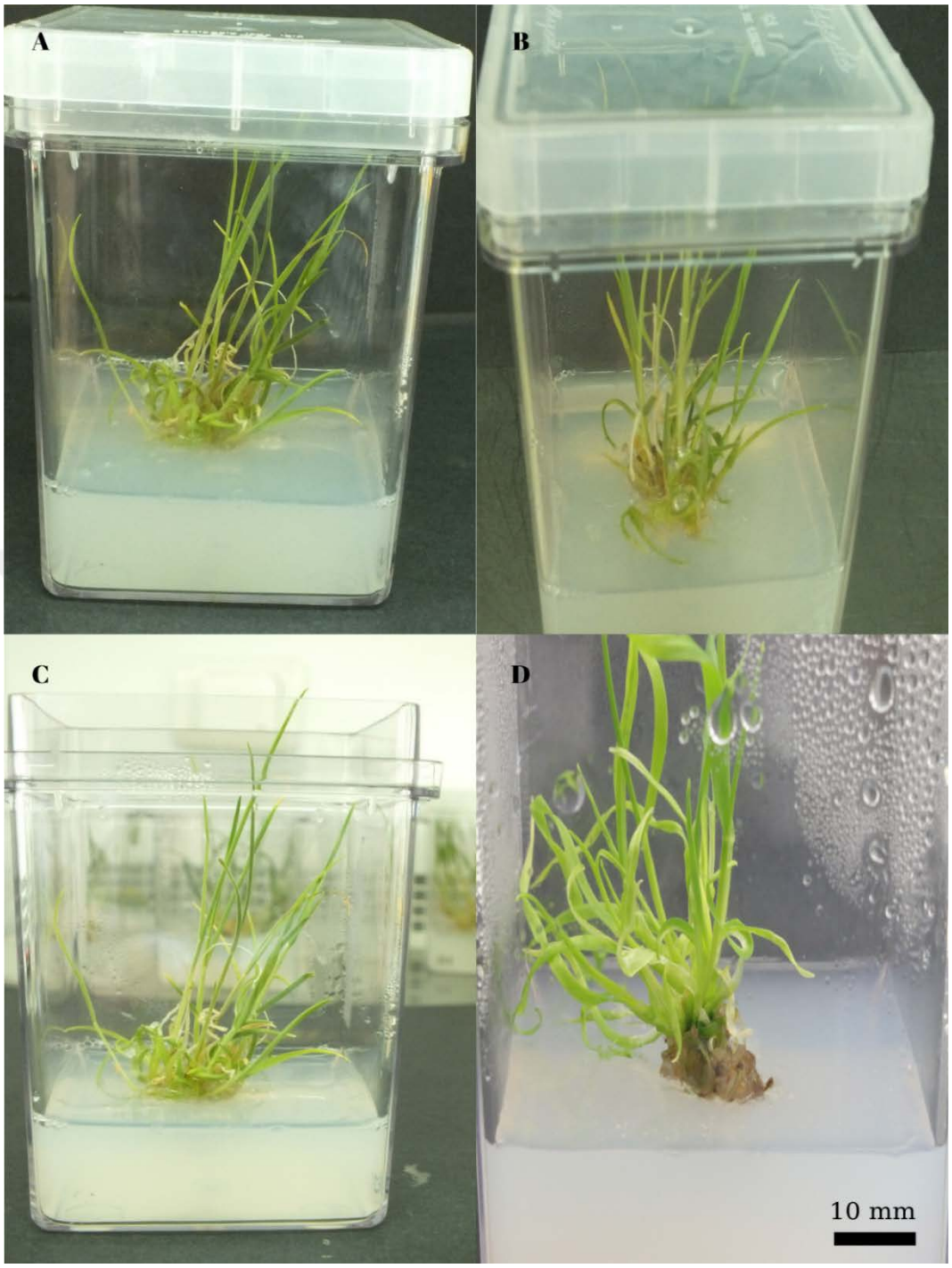

Figure 4.13. Bread wheat mature embryos forming regeneration. **A:** 0 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron. **C:** 2 mg/L BAP under 6.2 mg/L Boron. **D:** 2 mg/L BAP under 6.2 mg/L Boron.

4.5 Acclimatization

It is very difficult to transfer the plants in the culture to the soil and continue their life. Cultures must be acclimated with an intermediate period before transferring to the greenhouse or field. For plants grown in vitro conditions, there is an excessive loss of water when taken under normal conditions due to the open stomata, the number of stomata per unit area and the absence of a waxy structure in the cuticle layer. To prevent this, when the plant is taken into normal environment, it is practiced in an extremely humid transition period.

Plants showing sufficient shoot and root growth in vitro conditions were taken into pots containing sand: peat (1/3) to complete their development (Figure 4.14-20). Thanks to plastic bottles in the air-conditioning cabin under suitable conditions, acclimation is provided. Later, the plants were developed by making weekly watering processes.

Figure 4.14. Bread wheat whole plant. **A:** 2 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron

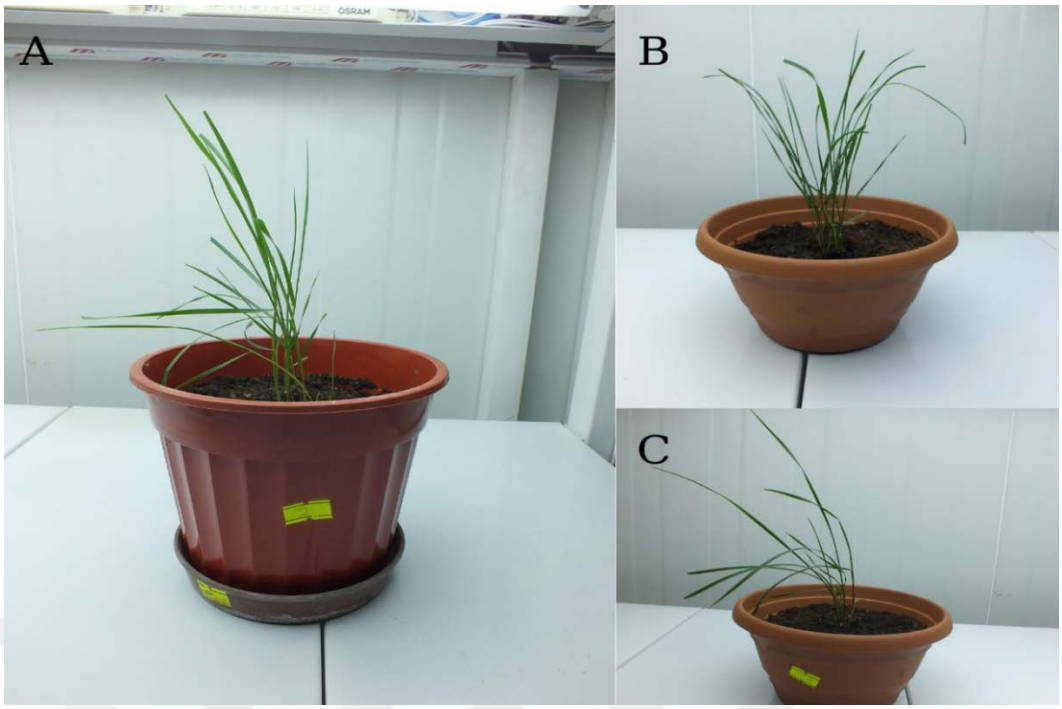

Figure 4.15. Einkorn wheat whole plant. **A:** 0 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron. **C:** 0.5 mg/L BAP under 6.2 mg/L Boron.

Figure 4.16. Bread wheat whole plant forming stages. **A:** 38 days (Node visible). **B:** 43 days (Second node visible). **C:** 49 days (Head visible). D**:** 60 days (Flowering). **E:** 90 days (Full ripe).

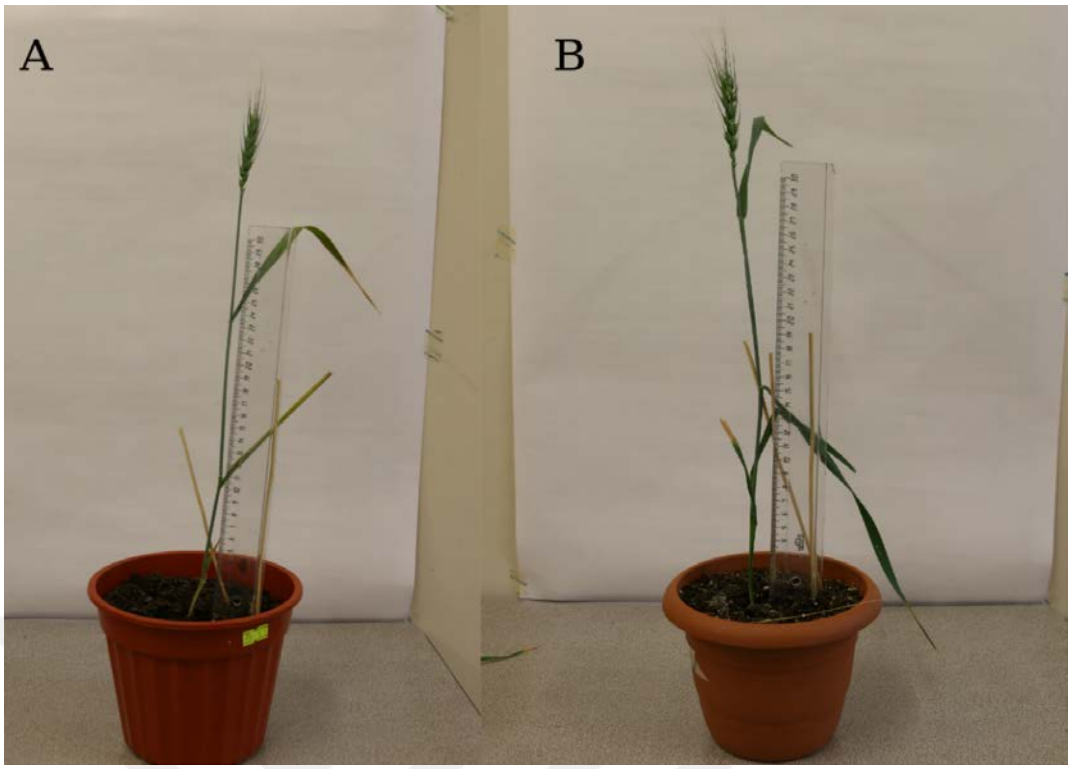

Figure 4.17. Bread wheat whole plant. **A:** 0 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron.

Figure 4.18. Breads wheat anthesis.

Figure 4.19. Bread wheat anthesis.

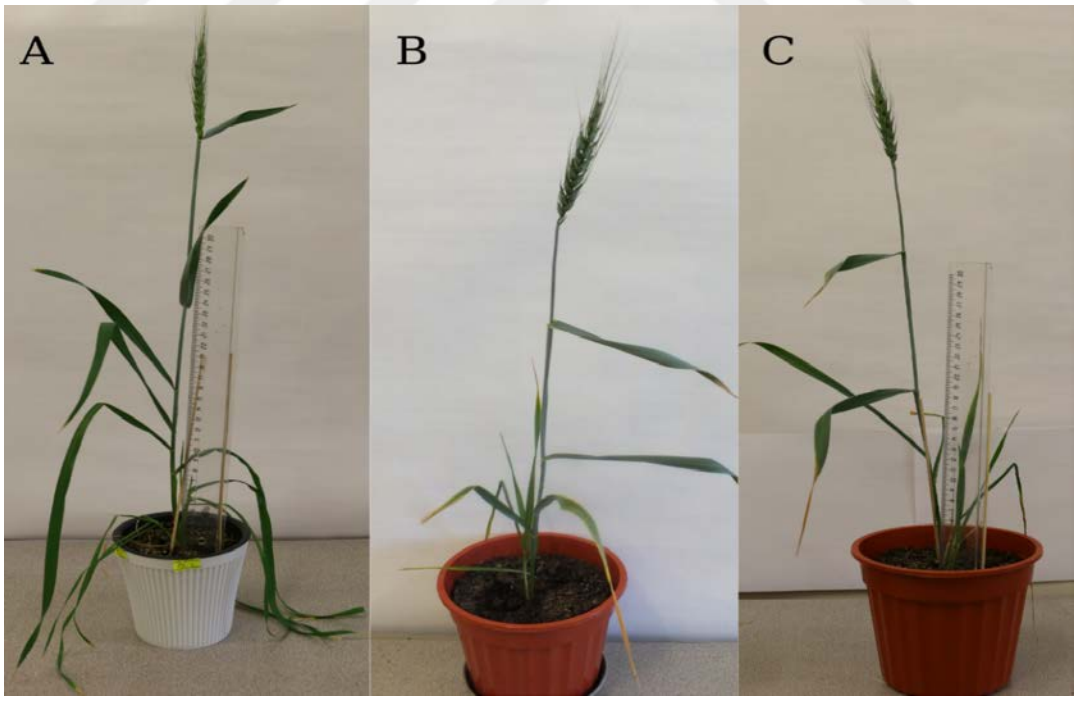

Figure 4.20. Bread wheat whole plant. **A:** 2 mg/L BAP under 6.2 mg/L Boron. **B:** 2 mg/L BAP under 6.2 mg/L Boron. **C:** 0.5 mg/L BAP under 6.2 mg/L Boron.

5. DISCUSSIONS

The problem of feeding the world population, which is growing rapidly under changing environmental conditions, increases the importance and value of wheat. Besides the population increase, the drought problem caused by global warming preventing the necessary nutritional needs. Wheat contributes from different economic aspects besides nutrition. The use of the agricultural lands needed plays a role in increasing agricultural production and country income.

Soils affected by Boron toxicity in the world have a significant percentage. The soil is usually seen in the issue of boron toxicity in Turkey. 20% of the lands where grain is produced have a toxic level of boron for cereals (Goldbach and Huang, 2010). One of the main objectives of the studies on boron toxicity is to develop more efficient and highly tolerant plants in these areas. For this reason, the development and dissemination of effective and highly successful new species is of great importance. On the other hand, Boron is an essential element for plant growth and development (Siddiqui et al, 2013). It has been determined that by giving boron with fertilizer in wheat with boron deficiency, it increases yield and quality (Wrobel, 2009). The amount of boron needed in plants is very low. In case of excess boron application, it has been reported that it causes a decrease in shoot height and shoot age weight (Marschner, 1995). Application of boron to soil is a common method and it is not used much in tissue culture environment. *In vitro* wheat embryos grown in artificially controlled nutrient media can be a good material for boron stress. In MS nutrient media developed by Murashige and Skoog (1962), element concentrations were adjusted in appropriate amounts according to the needs of the plant. Ambient pH value is adjusted appropriately for plants, thus preventing pH problems.

Today, biotechnological methods have been intensively used to solve the problems encountered in classical plant breeding methods. By using biotechnological methods; plant gene resources that are resistant to biotic and abiotic stress factors such as disease, pest and drought are increasingly being used in nature.

In this study, embryo culture was performed by applying 2, 4-D and Dicamba plant growth regulators under different boron concentrations to mature embryos of proprietary Tosunbey wheat and IZA (einkorn) grown in Seben / Bolu.

In the study, wheat embryos were separated from the endosperms and formed callus in an appropriate media. Weights, dimensions, and forming rates of callus formed were observed. The calli formed were then moved to regeneration environments containing plant regeneration and the BAP plant growth regulator to obtain a complete plant. Regeneration capacity and number of plantlets were determined.

Some differences seen during the research are as follows; (i) callus formation in einkorn wheat begins within 3-4 days, while bread wheat begins within 7-8 days, (ii) white roots appear on callus in bread wheat, but not in einkorn, (iii) regeneration rate is higher in bread wheat than einkorn wheat, (iv) plants formed in bread wheat are longer than einkorn wheat, (v) callus formation percentages in einkorn and bread wheat were 100%. Since there is no difference between the obtained values, the results are not included in the sections.

It is known that genotype is the most important factor for success in gene transfer in wheat, callus formation and plant regeneration (Şehirali and Özgen, 1998). Additionally, genotype is the most important factor for success in gene transfer in wheat, callus formation and plant regeneration (Aydın et al, 2009). In this study, it was determined that the effect of genotype on callus formation, callus weight and callus diameter is very important (Zale et al, 2004; Ahmet and Adak, 2007; Khan et al, 2015). Similar findings were obtained in other studies on this subject. Plant regeneration capacity and its effect on the number of plantlets did not show results as in other studies.

As a result of the research, when the genotypes were compared, the highest callus weight, callus diameter and regeneration capacity occurred in einkorn (IZA), while the highest number of plantlets was determined in bread wheat (Tosunbey) wheat. The probable reason why the species are different in terms of the factors examined is because that the chromosome numbers and hormone levels they contain are different. Bharskaran and Smith, (1990) explained that the differences between genotypes in terms of callus formation and plant regeneration may result from differences in the level of hormones they contain.

As a result of the research, the highest callus weight in einkorn was determined to be 3.71 g in an environment containing 1 mg/L Dicamba under 6.2 mg/L Boron stress and 3.46 g in an environment containing 4 mg/L Dicamba in Bread wheat under 12.4 mg/L boron stress. In Malik et al. (2003)'s experiment, this value was found to be 3.70 g and 3.60 g for Inqilab-91 and Pavon-76, which is bread

wheat. In another study, it was attempted to create callus in different cultures of different kinds of wheat mature embryos in culture prepared using 2,4-D. In the study, they stated that the highest callus weight was 5.7 g in Yakar variety, which is bread wheat, and 4.3 g in Altın variety, which is durum wheat. It can be thought that the results obtained in our study were less than the results of this study because of the boron toxicity applied and the genotype used. Another reason may be the types and amounts of auxin used. The most used types of auxin in mature embryo culture of wheat and other cereal types are 2,4-D, Dicamba and Picloran (He and Lazzeri, 2001; Satyavathi et al. 2004; Filippov et al 2006). It is determined that Dicamba, which is used differently from this study, is more effective than 2,4-D (Mendoza and Kaeppler 2002). It is stated that Dicamba is used faster by metabolism and therefore increases callus formation. In contrast, 2,4-D resists enzymatic degradation and aggregation. Therefore, it can remain highly stable in plant cells (Papenfuss and Carmen, 1987; Moore, 1989).

In this study, callus dimensions were found to be 1.10 cm in einkorn wheat in the media containing 1 mg/L Dicamba under the stress of 12.4 mg/L boron, and 1.22 cm in the environment containing 4 mg/L Dicamba in the bread wheat under the stress of 6.2 mg/L. In their study, Rashid et al. (2009) found the highest callus diameter among the 4 different bread wheat varieties as 2.05 cm in a media 5 mg/L 2,4-D + Kinetin. The absence of any stress factor in this study is likely to be effective in high results. Although the hormones used are the same, the stress factor may be different from the wheat varieties used in the research.

For maturation of embryogenic calli and plant regeneration, embryogenic calli are transferred to environments with no hormone or low cytokinin levels. Cytokinins used in these environments are used with auxins or alone (Schulze, 2007). The most commonly used cytokinins are BAP, kinetin, zeatin and TDZ (He and Lazzeri, 2001). In this study, media that containing BAP plant growth regulator, a type of cytokine, were prepared to provide regeneration activity of calli obtained after callus formation. It was found that there was no significant difference in the regeneration of wheat species in calli transferred to the media. Boron stress showed a significant difference. The highest regeneration capacity was 71.3% in the media containing 0 mg/L BAP under 6.2 mg/L boron stress in einkorn and 65.3% in the media containing 0 mg/L BAP under 6.2 mg/L Boron stress in Bread wheat. The lowest regeneration capacity was found to be 6% in the environment containing 2

mg/L BAP at 37.2 mg/L Boron stress in einkorn and 7.33% in the environment containing 0.5 mg/L BAP under the stress of 24.6 mg/L Boron in wheat. In the study conducted by Rashid et al. (2009), the highest regeneration capacity was observed in Inqilab-91 bread wheat with 71.75% in the media containing 2.5 mg/L BAP + 0.1 mg/L IAA. Many studies have reported that mature embryos with endosperm support have better plant regeneration capacity than mature embryos without endosperm support (Chen et al, 2006). In the study conducted by Rashid et al. (2009), we have achieved the results obtained by using mature endosperm-supported embryos using mature embryos. It is thought that this is due to the fact that boron is an essential element for plant growth and development.

When wheat species and boron stress conditions were compared in terms of number of plants; the highest number of plantlets was determined in the environment containing 0.5 mg/L BAP under the stress of 3.71 and 6.2 mg/L Boron, and the average number of the lowest number of plants was 1.29 in the environment containing 0.5 mg/L BAP under the stress of 24.6 mg/L Boron in wheat. In a study by Ahmed and Sagi (1993), they obtained a large number of embryogenic callus using immature embryos of bread wheat. They established embryogenic cell suspension cultures with the calli they obtained. They kept the callus with embryogenic capacity for more than 6 months and provided regeneration. They added IAA and BAP to the media for regeneration. At the end of the experiment, plant regeneration and green shoots were detected in calli. They found the results in the mature embryo we used in immature embryos.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study, using different hormones and concentrations under boron stress, it was ensured that callus formation and regeneration system was successfully obtained in mature embryo culture of two different wheat species. In this study, it can be concluded that it is possible to prefer mature embryos in cases where it is not possible to use immature embryos due to the success in wheat mature embryo culture. In terms of callus weight and diameter, it can be recommended to use einkorn wheat in terms of plant regeneration capacity and number of plantlets in the tissue and embryo culture studies of bread wheat. In previous studies, comparing different hormones under boron stress of wheat plant is almost nonexistent. In addition, diploid einkorn and hexaploid bread wheat were not compared under boron stress. In terms of boron stress, as the amount of boron increases, negativities occur in tissue culture. It has been shown that keeping the amount of boron at a normal level is better for plant tissue culture. Therefore, it is anticipated that this study would shed light on other future studies.

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1. Mehmet ÖRGEÇ, Fatma PEHLİVAN KARAKAŞ, Günce ŞAHİN, Ferdi AĞIL, Nusret ZENCİRCİ. 2018. "Einkorn (Triticum monococcum ssp. monococcum) in vitro propagation sterilization protocol", Secondary Metabolite. International Journal, 5(2), 67-74.
2. Noreen ASLAM, Cisem Nildem DOGAN, Ferdi AGIL, Huri Melek YAMAN, Nusret ZENCİRCİ. 2019. "Conservation Of Plant Genetic Resources For Rare And Endangered Species In Turkey", Uluslararası Anadolu Ziraat Mühendisliği Bilimleri Dergisi (International Journal of Anatolian Agricultural Engineering Sciences), 1(1), 7-13.

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1. Ferdi AĞIL, Sandeep Kumar VERMA, Fatma PEHLİVAN KARAKAŞ, Mehmet ÖRGEÇ, Nusret ZENCİRCİ. 2018. "Embryo Cultures of IZA (Einkorn) Wheat (Triticum monoccocum ssp. monoccoccum) under Boron Toxicity", Uluslararası 4. Tıbbi ve Aromatik Bitkiler Sempozyumu (4th International Symposium of Medicinal and Aromatic Plants), Poster Presentation, Çeşme, İzmir, Turkey.
2. Ferdi AĞIL, Fatma PEHLİVAN KARAKAŞ, Sandeep Kumar VERMA, Mehmet ÖRGEÇ, Nusret ZENCİRCİ. 2018. "Embryo Cultures of Bread Wheat (Triticum aestivum) under Boron Toxicity (Bor Toksisitesi Altında Ekmeklik Buğdayın (Triticum aestivum L.) Embriyo Kültürüne Alınması)", Türkiye Yerel Buğdayları Sempozyumu (Turkey Local Wheats Symposium), Poster Presentation, Bolu, Turkey.

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