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

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Abstract

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

Key message

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

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

Introduction

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

The diploid (2n = 2x = 14, A\(^{m}\)A\(^{m}\)) cultured einkorn (*Triticum monococcum* ssp. *monococcum*) is one of the first cultivated wheat. It is derived from *T. boeticum* in the Karacadağ mountain in Southern Eastern Turkey, about 10,000 to 13,000 years ago (Peng et al. 2011). Einkorn contains high amounts of carotenoids, antioxidants, vitamins (B and E), and minerals such as zinc, iron, and copper (Suchowilska et al. 2012; Şahin et al. 2018; Yaman et al. 2019; Pehlivan-Karakaş et al. 2020). Its protein content ranges between 16.00 and 28.50% (Hammer et al. 1996). Moreover, einkorn wheat has also useful genetic traits such as resistance to biotic/abiotic stresses (Abdel-Aal et al. 1995). These genetic traits may be advantageous in wheat breeding programs. The other important cultivated wheat species are tetraploid (2n = 4x = 28, AABB) durum (*T. durum*) and hexaploid (2n = 6x = 42, AABBDD) bread (*T. aestivum* L.) wheats. These two are the most grown and consumed wheat species today.
Global crisis such as Covid-19 pandemic showed that crop production such as wheat, rice maize etc. is highly strategic for food supply and safety. Humankind may face a global warming crisis in near future. Global warming may, unfortunately, worsen climatic conditions, deteriorate agricultural estate conditions, decrease quality and production of the crops. To feed huge amount of human population today, new approaches should be developed to improve high yielding and biotic/abiotic stress resistant crops (Örgeç et al. 2021).

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

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

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

Material and methods

Seed source

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

Seed sterilization

De-hulled einkorn and bread wheat seeds were washed by five drops Tween 20 (Merck, Darmstadt, Germany) and five different boron concentrations ranging from 0 to 25 ± 1 °C under dark conditions for four weeks. Callus weight of calli formed after 4 weeks were measured and recorded. Five replicates. The culture media were kept in the growth room at 25 ± 1 °C under dark conditions for four weeks. Callus weight of calli formed after 4 weeks were measured and recorded.

Plant regeneration

Calli derived from immature seeds of einkorn and bread wheats were carried to regeneration media. The media composed of 4.4 g L⁻¹ MS, 30 g L⁻¹ sucrose, 8 g L⁻¹ agar and five different boron concentrations ranging from 0 to 25 °C.
37.2 mg L\(^{-1}\) were supplemented with different BAP (6-Benzylaminopurine) (Duchefa-Netherlands) concentrations ranging from 0 to 2 mg L\(^{-1}\) for four weeks. The culture was kept in a growth room at 25 ± 1 °C under 16-h light/8-h dark photoperiod. Subcultures were performed every two weeks. At the end of four weeks, regenerating calli in petri were counted for number of plantlets.

**Acclimatization**

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

**Statistical analysis**

The experiment was carried out in a five-replicate randomized complete plot design with n ten embryos contained in petri dishes. Data on callus weight (g), and number of plantlets (n) were obtained. Statistical analyses were performed by using SPSS 23.0 program. The analysis of variance (ANOVA) with Duncan post hoc test was used to determine the differences between boron doses, plant growth regulators, and species (Düzgüneş et al. 1987; Saha et al. 2017).

**Results**

**Callus weight (g)**

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

Among two different auxins, dicamba responded better than 2,4-D for callus weight. One mg L\(^{-1}\) dicamba significantly produced the highest callus weight (3.71 ± 0.13 g) for einkorn wheat cultured on MS medium supplemented with 6.2 mg L\(^{-1}\) boron. On the other hand, for bread wheat, 4 mg L\(^{-1}\) dicamba cultured on MS medium with 12.4 mg L\(^{-1}\), boron responded better than all other hormone doses for the callus weight (3.46 ± 0.40 g) (Table 1).
Variance analysis showed that there was a significant interaction between wheat genotype/boron dose, wheat genotype/boron stress, and boron stress/hormone on callus weight. However, there was no significant interaction for hormone type, wheat genotype/hormone type and wheat genotype/boron stress/hormone type at 1 mg L\(^{-1}\) hormone dose (Table 2). As seen in Table 3, there was significant interaction between wheat genotype and boron stress and hormone type and boron stress/hormone type for callus weight. Wheat genotype/boron stress and wheat genotype/hormone type, and wheat genotype/boron/hormone type did not significantly interact at 2 mg L\(^{-1}\) hormone dose.

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

### Plantlets number (n)

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

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

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

### Acclimatization

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

### Discussions

Optimum media composition was 1 mg L\(^{-1}\) dicamba with 6.2 mg L\(^{-1}\) boron for callus weight, and 2 mg L\(^{-1}\) BAP for plantlet numbers in einkorn (*Triticum monococcum* ssp. *monococcum*). On the other hand, optimum media content was 4 mg L\(^{-1}\) dicamba with 12.4 mg L\(^{-1}\) boron for callus weight, and 0.5 mg L\(^{-1}\) BAP with 6.2 mg L\(^{-1}\) boron for plantlet numbers in bread wheat (*Triticum aestivum* L.). Moreover, dicamba was superior than 2,4-D on callus induction for both wheat species.

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

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

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

The results here showed that callus weight, genotype and boron stress were quite significant at all 2,4-D and Dicamba doses. Species varied for callus weight. Einkorn callus was heavier than that of bread wheat. This result was supported by Nasircilar et al. (2006), where five different bread and five durum wheat callus weights differed. Both studies indicated that genotype may play an important role in callus weight. Boron dose is another important factor which...
Table 1  Effects of different 2,4-D and Dicamba concentrations under boron stress on callus

| Genotypes                        | Boron Concentrations (mg L\(^{-1}\)) | Hormone Types | Hormone Concentrations (mg L\(^{-1}\)) | Callus Weight (g)* |
|----------------------------------|--------------------------------------|---------------|----------------------------------------|-------------------|
| Einkorn (\(T. monococcum\)      | 0                                    | 2, 4-D        | Control (0)                            | –                 |
| sssp. monococcum) (IZA)          |                                       | 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   | abcd                                   |                   |
|                                  | 2                                     | 3.50 ± 0.30 abcd    | abc                                     |                   |
|                                  | 4                                     | 2.70 ± 0.26 fghijkl  | abc                                     |                   |
| 6.2                              | 2, 4-D                                | Control (0)    | –                                      |                   |
|                                  | 1                                     | 3.54 ± 0.35 abc    | abc                                     |                   |
|                                  | 2                                     | 3.03 ± 0.55 cdefghi | cdefghi                                |                   |
|                                  | 4                                     | 2.88 ± 0.60 efgijkl | fghijkl                                 |                   |
|                                  | Dicamba                               | Control (0)    | –                                      |                   |
|                                  | 1                                     | 3.71 ± 0.13 a      | abcd                                    |                   |
|                                  | 2                                     | 3.54 ± 0.32 abcd   | abc                                     |                   |
|                                  | 4                                     | 3.64 ± 0.16 ab      | ab                                      |                   |
| 12.4                             | 2, 4-D                                | Control (0)    | –                                      |                   |
|                                  | 1                                     | 3.34 ± 0.56 abcde  | abcde                                   |                   |
|                                  | 2                                     | 2.59 ± 1.47 ghijklmn| ghijklmn                                |                   |
|                                  | 4                                     | 2.23 ± 0.12 mnopqrs | mnopqrs                                |                   |
|                                  | Dicamba                               | Control (0)    | –                                      |                   |
|                                  | 1                                     | 2.92 ± 0.51 ddefghi| defghi                                  |                   |
|                                  | 2                                     | 2.43 ± 0.46 hijklmno| hijklmno                                |                   |
|                                  | 4                                     | 2.17 ± 0.26 ijmnopqr| ijmnopqr                               |                   |
| 24.6                             | 2, 4-D                                | Control (0)    | –                                      |                   |
|                                  | 1                                     | 2.57 ± 0.26 ghijklmn| ghijklmn                                |                   |
|                                  | 2                                     | 2.32 ± 0.44 klmnopq | klmnopq                                 |                   |
|                                  | 4                                     | 2.29 ± 0.69 mnopq   | mnopq                                   |                   |
|                                  | Dicamba                               | Control (0)    | –                                      |                   |
|                                  | 1                                     | 2.02 ± 0.39 nopq    | nopq                                    |                   |
|                                  | 2                                     | 2.04 ± 0.25 nopq    | nopq                                    |                   |
|                                  | 4                                     | 2.34 ± 0.37 jklmnopq| jklmnopq                               |                   |
| 37.2                             | 2, 4-D                                | Control (0)    | –                                      |                   |
|                                  | 1                                     | 2.32 ± 0.24 klmnopq | klmnopq                                |                   |
|                                  | 2                                     | 2.36 ± 0.26 jklmnopq| jklmnopq                               |                   |
|                                  | 4                                     | 1.99 ± 0.17 nopq    | nopq                                    |                   |
|                                  | Dicamba                               | Control (0)    | –                                      |                   |
|                                  | 1                                     | 2.29 ± 0.29 lmnopqr | lmnopqr                                 |                   |
|                                  | 2                                     | 2.42 ± 0.47 hklmnopq| hklmnopq                               |                   |
|                                  | 4                                     | 2.12 ± 0.55 mnoq    | mnoq                                    |                   |
| Bread Wheat (\(T. aestivum\)     | 0                                    | 2, 4-D        | Control (0)                            | –                 |
| L.\) (cv Tosunbey)               |                                       | 1             | 1.97 ± 0.44 nopq                       |                   |
|                                  |                                       | 2             | 2.14 ± 1.02 mnopq                      |                   |
|                                  |                                       | 4             | 1.77 ± 0.35 pqrst                      |                   |
Table 1 (continued)

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

*Different letters indicate significant differences at p < 0.05
### Table 2
Analysis of variance of callus weights at 1 mg L\(^{-1}\) 2,4-D/Dicamba in mature wheat embryo

| Source                              | Sum of Squares | Df | Mean Square | F       | Sig  |
|-------------------------------------|----------------|----|-------------|---------|------|
| Genotypes                          | 12.236         | 1  | 12.236      | 77.218  | 0.000|
| Boron Stress                        | 10.466         | 4  | 2.616       | 16.513  | 0.000|
| Hormone Type                        | 0.509          | 1  | 0.509       | 3.217   | 0.076|
| Genotypes X Boron stress            | 3.736          | 4  | 0.934       | 5.894   | 0.000|
| Genotypes X Hormone types           | 0.164          | 1  | 0.164       | 1.040   | 0.310|
| Boron stress X Hormone types        | 3.734          | 4  | 0.933       | 5.891   | 0.000|
| Genotypes X Boron Stress X Hormone types | 0.549       | 4  | 0.137       | 0.867   | 0.487|
| Error                               | 12.676         | 80 | 0.158       |         |      |
| Total                               | 42.848         | 99 |             |         |      |

### Table 3
Analysis of variance of callus weights at 2 mg L\(^{-1}\) 2,4-D/Dicamba in mature wheat embryos

| Source                              | Sum of Squares | df | Mean Square | F       | Sig  |
|-------------------------------------|----------------|----|-------------|---------|------|
| Genotypes                          | 3.964          | 1  | 3.964       | 19.238  | 0.000|
| Boron Stress                        | 10.119         | 4  | 2.530       | 12.277  | 0.000|
| Hormone Type                        | 5.424          | 1  | 5.424       | 26.324  | 0.000|
| Genotypes X Boron stress            | 1.310          | 4  | 0.328       | 1.590   | 0.185|
| Genotypes X Hormone types           | 2.283          | 1  | 2.283       | 11.080  | 0.001|
| Boron stress X Hormone types        | 4.796          | 4  | 1.199       | 5.818   | 0.000|
| Genotypes X Boron Stress X Hormone types | 1.780        | 4  | 0.445       | 2.160   | 0.081|
| Error                               | 16.485         | 80 | 0.206       |         |      |
| Total                               | 46.161         | 99 |             |         |      |

### Table 4
Analysis of variance of callus weights at 4 mg L\(^{-1}\) 2,4-D/Dicamba in mature wheat embryos

| Source                              | Sum of Squares | df | Mean Square | F       | Sig  |
|-------------------------------------|----------------|----|-------------|---------|------|
| Genotypes                          | 1.212          | 1  | 1.212       | 5.585   | 0.021|
| Boron Stress                        | 8.235          | 4  | 2.059       | 9.486   | 0.000|
| Hormone Type                        | 19.740         | 1  | 19.740      | 90.955  | 0.000|
| Genotypes X Boron stress            | 3.382          | 4  | 0.846       | 3.896   | 0.006|
| Genotypes X Hormone types           | 11.868         | 1  | 11.868      | 54.683  | 0.000|
| Boron stress X Hormone types        | 0.776          | 4  | 0.194       | 0.894   | 0.471|
| Genotypes X Boron stress X Hormone types | 0.917        | 4  | 0.229       | 1.057   | 0.383|
| Error                               | 17.363         | 80 | 0.217       |         |      |
| Total                               | 61.802         | 99 |             |         |      |
affected callus weight in our study. Einkorn wheat resulted in the highest callus weight under 6.2 mg L\(^{-1}\) boron while bread wheat did it under 12.4 mg L\(^{-1}\). Bread wheat was more resistant at high boron concentration than einkorn wheat. Moreover, callus weight decreased when boron concentration increased in the culture media. The study by Huang and Graham (1990) with seven different wheat cultivars under six different boron concentrations on cellular and organ level was also parallel with our research. In our report, only two and four mg L\(^{-1}\) hormone doses significantly affected callus weight than other hormone doses. In our study, one mg L\(^{-1}\) dicamba containing 6.2 mg L\(^{-1}\) boron for einkorn wheat and four mg L\(^{-1}\) dicamba containing 12.4 mg L\(^{-1}\) boron were the best medium for callus weight. From that point of view, it appeared that dicamba gave better results in wheat mature embryo culture than 2,4-D. This was supported by Murin et al. (2012) with 26 wheat cultivars were cultured on callus media containing dicamba and 2,4-D.
The most important factor for plantlets number was the amount of boron in culture media. The best plantlets number was the culture media containing 12.4 mg L\(^{-1}\) boron for einkorn wheat and 6.2 mg L\(^{-1}\) boron for bread wheat. Einkorn wheat was more tolerant to boron stress than bread wheat. A previous study on in vitro of pear rootstock under salt-added boron stress, on the other hand, showed that the number of plantlets decreased with the increased boron and salt amounts in culture media (Sotiropoulos et al. 2006). Genotype-boron stress factor was significant only in culture media containing control group and 0.5 mg L\(^{-1}\) BAP. The best plantlet number in bread wheat was in culture media...
containing 0.5 mg L\(^{-1}\) BAP supplemented with 6.2 mg L\(^{-1}\) boron while control group containing 6.2 mg L\(^{-1}\) boron for einkorn wheat. Species did not affect the number of plantlets. Both wheat genotypes (Einkorn and bread wheat) were transported to the soil. However, bread wheat succeeded the following period better. At the end of 38 days, nodes started to be seen only in bread wheat. At the end of the acclimatization period, flowering (60 days) and ripening (90 days) happened in bread wheat. Einkorn wheat did not form spike. This may be an indication that bread wheat was better under acclimatization conditions.

### Conclusions

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

### Table 6

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

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

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