

Response of immature and mature embryos of modern Egyptian commercial durum (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.) for in vitro culture

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Abstract

Wheat is the most extensively cultivated and extremely nutritious cereal crop in the world. Increasing wheat productivity is essential to reduce the food gap in Egypt. Applying modern biotechnological plant breeding techniques could create new, highly productive cultivars. Tissue culture is a powerful tool that can be used to facilitate genetic transformation, induce genetic variation in plants, and create new and improved crop cultivars. The present study examines in vitro callogenesis expression and regeneration capacity of wheat cultivars in controlled laboratory conditions. Seeds from ten modern Egyptian commercial cultivars (Five durum and Five bread wheat cultivars) were collected for in vitro studies. Two different explants (immature embryos and mature embryos) and different media supplemented with different plant growth regulators were used to test the best wheat callus formation protocol. Immature embryos showed the highest callus formation value on MS medium supplemented with 2 mg/l 2,4-Dichlorophenoxy acetic acid, while mature embryos showed the highest callus formation value on MS medium supplemented with 3 mg/l 2,4-D. All 2,4-D supplemented media exhibited increased callus induction, suggesting that the 2,4-D as an effective growth regulator. The results of this study with modern Egyptian cultivars demonstrated that the response to tissue culture is greatly influenced by the genotype, the type of nutrient medium, and the interaction between them. The most effective explant source for callus indication and plant regeneration is immature embryos.

1. Introduction

Wheat, a self-pollinating annual crop, that is a member of the tribe Triticeae, genus *Triticum*, and family Poaceae (grasses), is the third-largest cereal produced globally, after rice and maize. Global wheat production in 2021 was 771 million tons, up 31% from 2000 [1]. It serves as an animal feed supply and a nutrient source for many people. Wheat contains more nutrients than any other dietary source for humans, including fats, minerals, vitamins, and protein. Wheat-based meals are more fiber-rich and healthy [2].

There are somewhere between 5-27 wheat species in the genus, depending on taxonomy [3], with tetraploid durum (*Triticum durum* Desf.) with genomes AABB [$2n = 4x = 28$] and hexaploid bread wheat (*Triticum aestivum* L.) with genomes AABBDD [$2n = 6x = 42$] being the most widely cultivated and consumed cultivars.

Bread wheat, a widely grown crop, is used in various products like pastries, cakes, rolls, cookies, and bread. It provides 20% of calories and is rich in protein, vitamins, and minerals. Durum wheat, the toughest endosperm, is grown exclusively for human consumption. Pasta products are also made from durum wheat[4]. Wheat can be used to make bread if it contains a high percentage of protein 13–16%. Low-protein wheat (8–11%) can

be used for biscuits, crackers, pastries, flatbreads, and noodles [5].

Wheat production in Egypt is low compared to annual consumption, causing economic problems. In 2021, Egypt produced 9.84 million tons but consumed 20 million tons. Egypt ranks first in importing wheat grains globally. Imports cost billions of dollars [1] and increasing land for wheat cultivation is challenging due to limited agricultural land. Vertical expansion by increasing its production unit cultivated area by developing new cultivars with high production capacity and a wide degree of adaptation and resistance to difficult environmental and biological conditions expansion can help to increase wheat production and also to increase wheat horizontal production by extending wheat growing outside the Nile Valley. Accepting these new cultivars for treatment and improvement using modern biotechnology methods has become a very important matter for any new genetic composition.

Wheat yield has significantly increased in recent years due to biotechnology [6]. In vitro culture and regeneration have been the focus of efforts to improve wheat. Plant tissue culture technology [7]is an essential component of biotechnology breeding and adds value to crop improvement programs. Three parameters are necessary for callus induction and in vitro plant regeneration: genotype, medium composition, and explant source [8]. Depending on the genotype of the wheat [10-9],

explants [12–11], culture medium [15–14–13], and growth regulators [16], several procedures can be done for tissue culture-based improvement of wheat. The tissue culture response varies among genotypes and cultivars, including callus induction, regeneration, and transformation efficiency [19–18–17].

The success of genetic engineering in crops is heavily influenced by a species' ability to regenerate and undergo callogenesis [21–20]. The current Egyptian wheat genotypes urgently need to be screened for tissue culture responsiveness.

The present study aims to evaluate the response of 10 new released commercial Egyptian durum and bread wheat cultivars for callus induction and plant regeneration using different explant sources on different media.

Materials and Methods

1. Experimental site

Grains of ten Egyptian wheat spring cultivars were grown in the Experimental Farm of Genetics Department, Faculty of Agriculture, Minia University, El-Minia, Egypt during the winter seasons of 2021/2022 and 2022/2023. In vitro culture was performed to assess the effective callusing and regeneration potentials of several wheat cultivars in the Biotechnology Laboratory in the Department of Genetics, Faculty of Agriculture, Minia University.

2. Plant Material and source

Five cultivars of commercial tetraploid spring Egyptian durum wheat (*Triticum durum* Desf.), namely, Beni Seuf 1, Beni Seuf 4, Beni Seuf 5, Beni Seuf 6, and Beni Seuf 7; as well as five cultivars of commercial hexaploid spring Egyptian bread wheat (*Triticum aestivum* L.), namely, Misr 1, Misr 2, and Misr 3, Giza 171, Sakha 95 were used in the current study. The grains of these ten Egyptian spring wheat cultivars were obtained from the Section of Wheat Research, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt (Table 1).

For callus induction and plant regeneration, two types of explants were used in this study (immature embryos, and mature embryos).

Table (1): Ten Egyptian spring wheat cultivars (5 durum and 5 bread cultivars) and their code were used in the present study.

Wheat cultivars	Code
Beni Seuf1	BS1
Beni Seuf4	BS4
Beni Seuf5	BS5
Beni Seuf6	BS6
Beni Seuf7	BS7
Misr1	M1
Misr2	M2
Misr3	M3
Giza171	G171
Sakha95	S95

3. Collection and Sterilization of the Explant's Surface

•Immature embryos

Immature embryos were obtained from immature seeds after pollination within 21–30 days. Temperature and other environmental factors affected how soon after pollination the embryos were selected. Immature seeds were surface sterilized in 70% ethanol for 1 min, then in 20% Clorox® solution (5% NaOCl) for 5 min, and then washed 4–5 times in sterile distilled water. After that, the seeds become ready for isolation of their immature embryos and culture. Five embryos were cultured on 200-ml jar containing 25 ml callus induction medium as one replicate, five replicates were used per medium. Culture of immature embryo explants was carried out as mentioned by Ozgen et al.[22].

•Mature embryos

Mature seeds were surface sterilized with 70% ethanol for 10 min, then 25% Clorox® solution (5% sodium hypochlorite) for 25 min; washed 4–5 times in sterile distilled water, and then soaked in sterile distilled water overnight at 25°C in darkness conditions. The seeds were sterilized again with 25% Clorox® for 15 min and then washed 4–5 times in sterile distilled water [24–23]. After that, the seeds become ready for isolation of their mature embryos and culture. Almost 8 cultured embryos /200-ml jar containing 25 ml callus induction medium were planted as one replicate, and three replicates were used per medium.

4. Culture media

•Callus induction media

Three different modified MS [25] media were used for callus induction from immature embryos (Table 2), and four different modified MS media were used for callus induction from mature embryos (Table 3). Each medium consists of 4.43 g/l MS powder medium (MS Media with macronutrients, micronutrients, vitamins, and glycine; Caisson Labs company, product number: MSP09-50LT.1; 836 South 100 East, Smithfield, UT 84335, USA), 30 g/l sucrose. Different concentrations of plant growth regulators were added (see below, Tables 2&3) and media adjusted to pH 5.8 by sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions and solidified with 0.8% Agar. Then each medium was sterilized in an autoclave at 121°C for 20 min and then stored in the darkness at room temperature for three days after sterilization to ensure the efficiency of sterilization and the absence of contamination. After that, the medium becomes ready to use. Then different explants cultures were incubated in an incubator (darkness) at 25 ± 2 °C for 3–4 weeks.

Table (2): MS media types and their content of plant growth regulators (mg) that were used in the experiment for callus induction from wheat immature embryos.

Media code	2,4-D*	Kin	IAA	NAA	BAP
MS1	2	—	—	—	—
MS2	4	1	—	2	—
MS3	2	1	—	2	—

*Note: 2,4-D= 2,4-dichlorophenoxy acetic acid; Kin= Kinetin; IAA= Indole-3-acetic acid; NAA= Naphthalene acetic acid; BAP= 6-Benzylaminopurine.

Table (3) :MS media types and their content of 2,4-D plant growth regulator (mg) that were used in the experiment for callus induction from wheat mature embryos.

Media	2,4-D concentration
MS1	2.5
MS2	3
MS3	4
MS4	5

•Regeneration media

➤ Durum wheat regeneration medium:

MSD regeneration medium was used, MSD medium consists of 4.43 g/l MS powder medium (Caisson Labs company, MSP09-50LT.1), 30 g/l sucrose, 0.5 mg/l IAA, 0.5 mg/l BAP, and adjusted to pH 5.8 by NaOH and HCl solutions then solidified with 0.8% Agar. Fifty ml of medium was poured in large glass jars. Media were sterilized in an autoclave at 121 °C for 20 min then stored in the dark for 3 days after sterilization to ensure the efficiency of sterilization. After that, the medium becomes ready to use. Five well-organized calli were subcultured on each jar as one replicate, five replicates were used. Cultures were growing under 16h light at 25 ± 2 °C for 3-4 weeks with one extra subculture.

➤ Bread wheat regeneration medium:

MSB regeneration medium consists of 4.43 g/l MS powder medium (Caisson Labs company, product number: MSP09-50LT.1; Smithfield, UT 84335, USA), 30 g/l sucrose, 0.8 mg/l NAA, 0.36 mg/l kinetin, and adjusted to pH 5.8 by NaOH and HCl solutions then solidified with 0.8% Agar. Fifty ml of medium was poured in large glass jars. Media were also sterilized in an autoclave at 121°C for 20 min. then stored in the dark for 3 days after sterilization to ensure the efficiency of sterilization. After that, the medium becomes ready to use. Five well-organized calli were subcultured on each jar as one replicate, five replicates were used. Cultures were growing under 16h light at 25 ± 2 °C for 3-4 weeks with one extra subculture.

5. Statistical analysis: -

I. Callus induction data

After 21-30 days from immature/mature embryo culturing, data were scored on the percentage of induced calli (number of explants formed calli per number of explants cultured on the medium X100).

II. Regeneration data

After 60 days from callus transferring onto the regeneration medium were recorded on the percentage of regenerated plants

(number of regenerable calli that produced plants per transferred calli differentiating X100). Also, the number of shoots per regenerable calli was calculated.

For Statistical analysis, all experiments were carried out in a completely randomized design with three replicates. Results were calculated as means and percentages, and analyzed with a one-way analysis of variance (ANOVA) conjoined by the least significant difference test ($P < 0.05$). Analysis of variance was calculated by using MSTAT computer program [26].

Results

Numerous elements have been found to have a significant impact on how different plant species respond to in vitro culture in prior studies. The impact of explants, wheat genotypes, and medium types on the process of callus induction and plant regeneration was examined in the current investigation with 10 modern commercial Egyptian wheat cultivars, characterized by abundant production and tolerance of harsh environmental conditions. Two distinct explants (immature embryos, and mature embryos) from commercial 5 Egyptian durum and 5 bread wheat cultivars were studied to assess their response for callus induction and plant regeneration.

1. Immature embryos

Immature embryos of ten wheat genotypes (five durum and five bread wheat cultivars) were cultured on three different media (MS1, MS2, and MS3) to determine the ideal medium type for callus induction and the best genotypes for callus formation.

1.1. Callus induction

Immature embryos of all examined ten wheat genotypes grown on the three-culture media were able to induce callus with different frequencies. Regarding their capacity to induce callus, Significant differences were found between the tested genotypes and genotype X medium interaction while no significant differences were observed between the tested three culture media (Table 4).

Table (4) The analysis of variance for the response of callus formation from the immature embryo culture of ten different wheat cultivars (5 durum wheat and 5 bread wheat) with three different types of callus induction media.

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F value	prob
Media(M)	2	114.813	57.407	0.916 9	
Genotype(G)	9	727.760	80.862	1.291 6**	0.248 4
G x M	18	2467.320	137.07 3	2.189 4**	0.006 5
Error	120	7512.800	62.607		
Total	149	10822.693			

The response of immature embryos of ten wheat genotypes is high on the three-culture media, therefore, there are differences in the speed of response, size of callus, and type of callus (Fig. 1A, B, C).

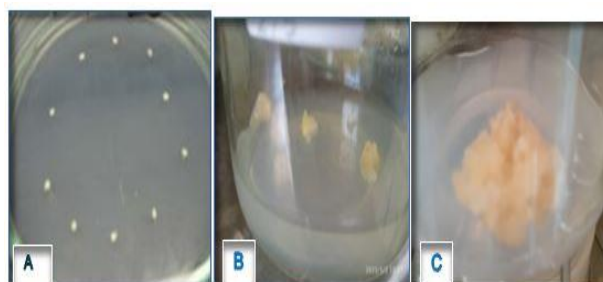


Figure (1): Steps of callus induction from immature embryos of ten wheat genotypes. (A): Isolated immature embryos of Misr 3 (21-day old) just culture on callus induction medium. (B): Initiated calli from cultured immature embryos of Misr 3 on callus indication medium, two-week in cultured. (C): Induced callus four-week grown of Beni Seuf 7 on MS1 callus induction medium.

Concerning the callus induction frequency of the ten Egyptian wheat cultivars, it was slightly different among most of the tested cultivars. The durum Beni Seuf 4, Beni Seuf 7, and bread Sakha 95 cultivars had the highest mean frequency (100%), while the durum Beni Seuf 1 cultivar was the least productive (93.33%). The callus induction levels of the remaining cultivars were intermediate (94.66% - 98.66%; Table 5).

Table (5) :The average percentages of callus formation from immature embryo cultures of ten different wheat cultivars (5 durum and 5 bread wheat) on three different modified MS media (MS1, MS2, and MS3).

Genotype	Number of cultured embryos /3 media	Callus induction % MS1	Callus induction% MS2	Callus induction% MS3	Mean of genotype
<i>Durum wheat</i>					
BS1	75	100 a	84 c	96 ab	93.33 b
BS4	75	100 a	100 a	100 a	100 a
BS5	75	100 a	84 c	100 a	94.66 ab
BS6	75	100 a	100 a	90 bc	96.66 ab
BS7	75	100 a	100 a	100 a	100 a
<i>Bread wheat</i>					
M1	75	100 a	100 a	89 bc	96.33 ab
M2	75	100 a	100 a	96 ab	98.66 ab
M3	75	92 abc	100 a	100 a	97.33 ab
G171	75	96 ab	100 a	100 a	98.66 ab
S95	75	100 a	100 a	100 a	100 a
Mean of cultured media	-	98.8 a	96.8 a	97.1 a	97.56

➤ Values followed by the same litter(s) are not significantly different.

➤ LSD value genotype medium interaction

0.05 5.721 3.133 9.908

The study found that using different concentrations of 2,4-D, Kin, and NAA in callus induction medium in this study has no significant impact on the frequency of callus formation in immature embryos. However, the MS1 medium (with 2,4-D 2.5 mg/l) produced the highest mean callus formation frequency across all genotypes (98.8%), then the MS3 medium (97.1%), while the MS2 medium produced only (96.8%; Table 5);

however, no significant differences noted between them (Table 5).

Respect to the impact of genotype of the tested cultivar on callus induction frequency on the different three callus induction media, it is clearly noted that the genetic background of the cultivar has considerable effects on callus induction frequency. Callus induction frequency of ten tested cultivars ranged from 93.33% (BS1 cultivar) to 100% (BS4, BS7, and S95 cultivars), significant differences were observed between cultivars (Table 5).

It is interesting that this data showed that the genotype X medium interaction had a significant impact on callus formation rates. For example, Beni Seuf 1, Beni Seuf 4, Beni Seuf 5, Beni Seuf 6, Beni Seuf 7, Misr 1, Misr 2, and Sakha 95 demonstrated the greatest values of immature embryo cultures' response (100%) for callus formation on MS1 medium. While Beni Seuf 4, Beni Seuf 6, Beni Seuf 7, and all 5 bread wheat (Misr1, Misr2, Misr 3, Giza171, Sakha 95) demonstrated the greatest values of immature embryo cultures' response (100%) for callus formation on MS2 medium. Whereas, Beni Seuf 4, Beni Seuf 5, Beni Seuf 7, Misr 3, Giza 171, and Sakha 95 demonstrated the greatest values of immature embryo cultures' response (100%) for callus formation on MS3 medium (Table 5).

BS1 and BS5 durum wheat cultivars showed the lowest ever callus formation percentage (84% both) on MS2 medium, followed by Misr1 bread wheat cultivar which showed the lowest callus formation percentage (89% on MS3 medium), also, Misr 3 bread wheat cultivar showed lowest callus induction (92%) on MS1 medium (Table 5).

Our observation in the present study found that Beni Seuf 5, 6, and 7 durum cultivars were the fastest and best in initiating callus formation, where their embryos were initiating calli within 3-4 days, unlike other cultivars which initiated calli during 7-14 days (Fig.1B).

On the other hand, it is noticed that the calli of BS1, BS4, BS7, BS6, BS5, M1, M2, M3, G171, and S95 cultivars were the biggest size on MS1 medium (Fig. 1C). While calli of BS1, BS4, BS6, M3, G171 and S95 cultivars were the smallest size on MS2 medium and also on MS3 medium. Whereas, the calli of BS5, M1, and M2 cultivars were intermediate in size on the MS2 medium and MS3 medium. The size of calli of the BS7 durum cultivar only was always large on all three types of callus induction media (MS1, MS2, and MS3 medium; Fig. 1C).

Calli of BS1, BS4, BS5, BS6, BS7, M1, M2, M3, G171 and S95 cultivars showed high callus induction frequency with embryogenic calli type on MS1 medium (Fig.2b). While calli of M1, M2, M3, G171 and S95 cultivars were non-embryogenic type on MS2 and MS3 medium (Fig.2a). On the other hand, calli of all durum cultivars (BS1, BS4, BS5, BS6, and BS7) were also embryogenic on MS2 and MS3 medium.

It is noticed that durum BS1, BS4, BS5, BS6, and BS7 cultivars are produced directly calli on the MS1 medium. While all bread M1, M2, M3, G171 and S95 cultivars produced calli with shoots on MS1 medium, but BS1, BS4, BS5, BS6, BS7, M1, M2, M3, G171 and S95 produced calli with shoots on MS2 medium and MS3 medium.

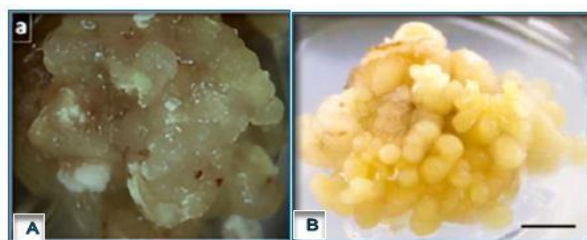


Figure 2: Callus morphology from the immature embryo culture of Misr1 cultivar on MS1 and MS2 medium.

(A): non-embryogenic callus of Misr1 cultivar on MS2 medium. (B): embryogenic callus of Misr1 cultivar on MS1 medium.

1.1.1. Plant regeneration and shoot formation

In this experiment, calli obtained from immature embryos of five different durum wheat cultivars on MS1 callus induction medium were subcultured onto MSD regeneration medium (MS + 0.5 mg/l BAP + 0.5 mg/l IAA). Similarly, calli of five different bread wheat cultivars obtained from immature embryos on MS1 callus induction media were also transferred to MSB regeneration medium (MS + 0.8 mg/l NAA + 0.36 mg/l Kin; Fig. 3 A, B, C, D, E). For plant regeneration study, we selected calli obtained on MS1 medium where this medium shows the highest percentage of callus induction, moreover, good quality, big size, and embryogenic type callus among the used 3 callus induction media.

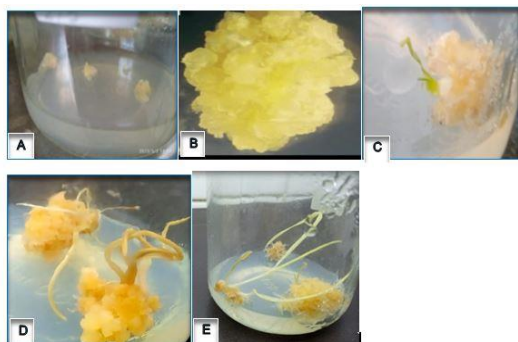


Figure 3: Plant regeneration from obtained calli of immature embryos of durum and bread wheat cultivars on MSD and MSB regeneration medium, respectively. (A): wheat calli of Misr3 cultivar were grown on a callus induction medium. (B): wheat calli of Misr 3 cultivar subcultured on MSB regeneration medium. (C): green shoots appear on wheat calli of Beni Seuf 5 on MSD regeneration medium. (D): shoots regenerated from 2 calli of Beni Seuf 7 on MSD regeneration medium. (E): complete wheat regenerated shoot of Misr 1 cultivar obtained *in vitro*.

Results of shoot regeneration from obtained calli on MS1 callus induction medium of ten Egyptian wheat cultivars were presented in Fig. (4). Significant variation was recorded for the parentages of regenerable calli as well as the percentage of number of regenerated shoots per callus between and amongst the ten tested genotypes (Fig. 4).

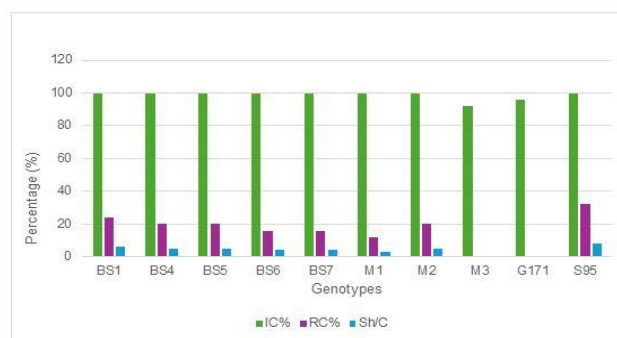


Figure (4): Mean percentage of induced callus (IC%) on MS1 callus induction medium, percentage of regenerated calli (RC%), and number of shoots per callus (Sh/C) of ten Egyptian wheat cultivars.

For the 5 durum wheat cultivars, the BS1 cultivar had the highest percentage of regenerated callus (24%), followed by the BS4 and BS5 cultivars (20%) and the BS6 and BS7 cultivars (16%). For the 5 bread wheat cultivars, the S95 cultivar showed the highest cultivar in regenerated callus (32%); while the M3 and G171 cultivars had zero regenerated calli, so it was the worst cultivars in regenerated callus, whereas the percentage of regenerated calli of M2 cultivar was 20% and the percentage of regenerated of M1 cultivar was 12%.

The study analyzed the number of regenerated shoots per callus of five durum wheat cultivars and showed the following results. The BS1 cultivar had the highest number of shoots (6 shoots per callus), followed by the BS4 and BS5 cultivars (5 shoots per callus), while the BS6 and BS7 cultivars showed the lowest cultivars in the number of shoots (4 shoots per callus). For the 5 bread wheat cultivars, the S95 cultivar had the highest number of shoots ever (8 shoots per callus), followed by the M2 cultivar had 5 shoots per callus and the M1 cultivar had 3 shoots per callus, while, the M3 and G171 cultivars had zero regenerated shoots, so it were the worst cultivars in the number of shoots too.

Generally, the S95 bread cultivar had the greatest value in callus induction (100%) on MS1 callus induction medium and the highest number of regenerated shoots per calli (8 shoots). While the M3 and G171 cultivar had a lower value in callus induction (92% and 96%, respectively) and zero regenerated shoots, the M1 and M2 cultivars had a greater value in callus induction (100%) on MS1 medium and regenerated shoots (3 and 5 shoots, respectively). On the other hand, all durum wheat cultivars had a greater value in callus induction (100%) on MS1 medium and regenerated shoots (4-6 shoots).

2. Mature embryos

Ten Egyptian wheat genotypes, five durum and five bread cultivars were used to perform *in vitro* culture of mature embryos. The embryos were isolated and cultured on four different types of callus induction media (MS1, MS2, MS3, and MS4; Table 2) to identify the best genotypes and medium types for callus formation.

2.1. Callus induction

To check the possibility of generating calli on mature embryos from ten wheat Egyptian genotypes that were investigated and

cultured on different callus induction media (MS1, MS2, MS3, and MS4). Their reactions to the in vitro culture of calluses varied greatly (Table 6).

The statistical analysis of obtained data of in vitro culture of mature embryos of the ten Egyptian cultivars shows significant differences between tested genotypes, media, and genotypes X media interaction as shown in ANOVA Table (6).

Table(6): The analysis of variance for the response of callus formation from the mature embryo culture of ten different Egyptian wheat cultivars (5 durum wheat and 5 bread wheat) grown on four different types of callus induction media.

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F value	prob
Media(M)	3	16395.217	5465.072	24.4013 ^{***}	0.0000
Genotype(G)	9	19973.533	2219.281	9.9090 ^{***}	0.0000
G x M	27	40662.783	1506.029	6.7243 ^{***}	0.0000
Error	80	17917.333	223.967		
Total	119	94948.867			

The data in Table (7) demonstrated that the Beni Seuf 5 (BS5) cultivar showed the highest mean callus induction frequency (40.54%) across all 3 tested media (MS1, MS2 and MS3), while the Misr1 (M1) cultivar was the least productive genotype (3.125%). The remaining wheat cultivars had intermediate callus induction percentages.

Also, the results in Table (7) showed that the used callus induction medium type significantly impacted the frequency of callus formation from mature wheat embryos. The MS2 medium was the most successful culture medium for forming calli from mature embryos, with the highest mean frequency (39.58%) of callus production for BS1, BS4, BS5, BS7, M2, and S95 cultivars, while MS1 medium had the lowest mean frequency of callus formation (6.63%). Whereas, MS3 (22.50%) and MS4 (13.25%) media produced calli at medium frequency.

The genotype X medium interaction significantly impacted callus formation rates. The BS5 cultivar had the highest response (41.33%) on the MS1 medium, while the M2 cultivar had the highest response (87.5%) on the MS2 medium. In contrast, the BS4 and the BS5 cultivars had the highest response (37.5%) for callus formation on the MS3 medium. The M3 bread cultivar had the highest response (75%) for callus formation on the MS4 medium.

Table (7): The percentages of callus formation from the mature embryo culture of ten different Egyptian wheat cultivars on four different modified MS media (MS1, MS2, MS3, and MS4). Note: not all ten cultivars were cultured on all 4-culture media.

Genotype	Number of cultured embryos/media	Callus induction% MS1	Callus induction% MS2	Callus induction% MS3	Callus induction% MS4	Mean
BS1	48	- *	33.33 def	12.5 fg	-	11.46 cd
BS4	48	-	29.17 ef	37.5 cde	-	16.67 c
BS5	72	41.33 bc **	83.33 a	37.5 cde	-	40.54 a
BS6	48	12.5 fg	45.83 cde	-	-	14.58 cd
BS7	72	-	50 cde	33.33 def	57.5 bcd	35.21 ab
M1	24	-	-	12.5 fg	-	3.125 d
M2	48	-	87.5 a	33.33 def	-	30.21 b
M3	48	-	54.17 bcd	-	75 ab	32.29 b
G171	24	-	-	29.16 ef	-	7.29 cd
S95	72	12.5 fg	12.5 fg	29.17 ef	-	13.54 cd
Mean	-	6.63 c	39.58 a	22.50 b	13.25 c	20.49

* -: not tested treatment.

**Values followed by the same litter(s) are not significantly different.

LSD value	genotype	medium	interaction
0.05	12.16	7.69	24.32

Generally, the mature embryos of most tested genotypes on four used callus induction media were slow in their response to form calli and needed a longer time to produce calli (one to two months) compared to immature embryos.

2.1.1. Plant regeneration

It is well established that a plant regeneration system is crucial for assessing the culture response of mature embryos.

Obtained calli from mature embryos of different durum wheat cultivars were transferred to MSD regeneration medium (MS + 0.5 mg/l BAP + 0.5 mg/l IAA). While calli from mature embryos of different bread wheat cultivars were transferred to MSB regeneration medium (MS + 0.8 mg/l NAA+0.36 mg/l Kin).

The results showed that mature embryos of ten wheat genotypes did not form any shoots on the two regeneration media which had zero regenerated calli, so that mature embryos were weak in their response to the plant regeneration system in modern Egyptian commercial wheat cultivars.

Discussion

This study focuses on the response of the immature and mature embryos of ten Egyptian commercial modern wheat cultivars for in vitro culture and reconnaissance on the role of genotype and culture medium and their interaction in this topic. The results show that callus induction rates from explanting wheat embryos are strongly influenced by the genotype, medium, and genotype X medium interaction. The MS1 callus induction medium (MS + 2 mg/l 2,4-D) was found to be the best medium for immature embryos of almost all cultivars due to their response rate, callus size, and callus type.

Murashige and Skoog solid medium (MS) supplemented with various combinations and concentrations of plant growth regulators was also found to be the best medium for wheat immature embryo culture[29-28-27].

Also, wheat genotypes significantly influence embryo culture response for callus induction capacity[30], as seen in bread and durum wheat. The interaction between genotype and medium

affects callus induction[19]. Auxins, particularly 2,4-D, are significant growth regulators in callus induction and proliferation, with 2 mg/l being the most effective concentration [30-36-35-34-33-32-31].

The study found that adding kinetin and naphthalene acetic acid beside 2,4-D to MS3 medium increased the capacity to form calli. The optimal medium for callus development for eight out ten tested genotypes was the MS2 medium, which contained 4 mg/l of 2,4-D. This finding is consistent with the observation of Yasmin et al., [19] that the medium with 4 mg/l of 2,4-D + Kin (1 mg/l) + NAA (2 mg/l) produced the greatest callus induction. Also, Mahmood et al.,[10] reached the same findings, where they discovered that the ideal 2,4-D concentration for callus induction percentage was 4 mg/l. While these findings contradict the findings of Haliloglu [37], who claimed that a concentration of 4 mg/l of 2,4-D demonstrated that highly hydrated calli was a common occurrence and that dead tissues were discovered in both the first and second embryonic phases. Data of Wernicke et al., [38], which showed that a high auxin content inhibited meristem cell division, are likewise consistent with our observations.

Previous studies on wheat in vitro culture have shown that genotype influences callus growth rate and frequency of plant regeneration [43-42-41-40-39]. Callus induction is a genotype-dependent process [9], and immature embryos are considered the most effective source of wheat tissue for significant plant regeneration. In vitro culture in wheat is also influenced by factors such as explant [45-44], genotype [46], and the medium used [48-47]. Our current study found that different genotypes of durum and bread wheat showed varying rates of callus induction, indicating that the induction of calli from wheat embryos is genetically regulated as has been previously documented by numerous research laboratories across the globe [50-49]. Arzani et al., [51] also noted that the potential for regeneration from immature embryos-derived calli varied significantly amongst cultivars.

Fennell et al.,[48] reported a decrease in wheat's capacity for regeneration after extended callus culture times, possibly due to the mutagenic action of auxins. Therefore, a brief callus culture period is critical for the effective transformation of modern wheat varieties.

The current study found that 2,4-D supplemented medium led to greater callus formation in embryogenic wheat callus formation, indicating that 2,4-D is an effective growth regulator which is consistent with earlier reports[54-53-52]. The study also revealed the effect of genotype on callus induction from wheat mature embryos. Despite immature embryos being the primary source of explants for wheat tissue culture, research now focuses on using mature embryos, which are more readily available and resistant to tissue culture.

This study confirms previous research showing low plant regeneration frequency in resistant cereal cultivars, attributed to low callus induction rate and physiological conditions. Mature embryos are popular for wheat in vitro cultures, but their regeneration potential is weak due to their high 2,4-D concentration or our used plant regeneration media were not suitable for regeneration process of these cultivars.

Conclusion

The current study shows that, for some modern Egyptian commercial wheat cultivars, high-frequency callus induction and shoot regeneration may be achieved by the use of immature embryos as an explant source. Wheat cultivars' potential for callogenesis and regeneration varies depending on their genotype and medium. Using a mixture of auxins and cytokinins in MS-based media can significantly increase the callusing and regeneration frequency of wheat cultivars. For wheat cultivars to experience maximum callus induction and plantlet regeneration, it is imperative to optimize these growth regulators, as well as the light and temperature conditions. Immature embryos are the best explants to induce calli and form shoots, as they are considered an ideal explant for inducing somaclonal variations, but mature embryos are the weakest explants in response to callus formation and plant regeneration.

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