Journal of Applied Biological Sciences 9 (1): 61-63, 2015
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Embryogenic Callus Propagation From *Triticum monococcum* ssp. monococcum Using Zygotic Embryos

Fethi Ahmet ÖZDEMİR[1]* Süleyman DOĞAN[1] Mehmet Uğur YILDIRIM[2] Nusret ZENCİRCİ[3]
[1] Department of Molecular Biology and Genetics, Faculty of Science, Bartın University, Bartın, Turkey
[2] Department of Field Crops, Faculty of Agriculture, Ankara University, Ankara, Turkey
[3] Department of Biology, Faculty of Arts and Science, Abant Izzet Baysal University, Bolu, Turkey

*Corresponding author:
Email: ozdemirfethiahmet23@yahoo.com

Received: February 20, 2015
Accepted: April 19, 2015

Abstract
This study reports a protocol about embryogenic callus propagation in *Triticum monococcum* ssp. monococcum using zygotic embryos. Embryogenic callus induction was noted on all concentrations of 2,4-D used in this study. Maximum embryogenical callus induction in terms of embryogenic callus weight was noted on MS medium containing 4 mg/l 2,4-D. Minimum embriyogenic callus weight was noted on MS medium containing 8 mg/l 2,4-D. Increase or decrease of 2,4-D concentratin resulted in decrease in embryogenic callus weight.
Keywords: *Triticum monococcum* ssp. monococcum, Callus, Zygotic embryos, 2,4-D.

INTRODUCTION

Cereals especially wheat constitute one of the basic constituents of nutrition since humans have passed to settled life. They are be produced in a variety of geographical and climatic conditions and both in form raw and processed material of many foods as indispensable part of human diet since thousands of years.

Einkorn (*Triticum monococcum* ssp. monococcum) wheat is glumed type diploid (2n=14), primitive wheat. This type of wheat has significant advantages in terms of phytochemicals (carotenoids, tocopherols, phenolic acids) and of the amount of protein and minerals, compared with commercially produced wheat types. Studies show that the carotenoid amount of einkorn wheat is approximately 2 - 4 times more compared to other wheat types. Its tocopherol content is also significantly higher than other species. It has 15.8 - 24.2% protein with 2.2 -2.5% ash is between while these values are 1.5 to 11.1% in common wheat. These results suggest that the species of einkorn wheat is quite rich in terms of protein and mineral contents [5; 6]. People with celiac disease cannot consume wheat gluten, barley and rye that contain proteins of similar structure. Nowadays the only treatment for these patients is a gluten free diet [10]. Some previous studies point out that einkorn wheat (*T. monococcum*) has an amino acid sequence of α – gliadin that causes celiac disease (gluten intolerance) [4; 17, 20]. But there are other studies that emphasise that some sorts of einkorn has less toxicity or no toxicity at all [3; 16; 18]. In recent years there have been intensive studies on *T. monococcum* especially due to its diploid nature. Moreover, the information obtained from study of this plant can easily be applied to bread durum and bread wheat in future.

This study aimed to contribute to future studies to breed einkorn wheat and producing embryogenic callus from zygotic embryos that is one of the ancient wheat species of Turkey.

MATERIALS AND METHODS

Einkorn wheat seeds used in this study were obtained from Abant Izzet Baysal University, Faculty of Arts and Science, Department of Biology. For easy removal of the zygotic embryos from the seeds after sterilization, the seeds were kept in sterile distilled pure water for 24 hours. After the seed husks were peeled they were sterilized treating them with commercial bleach (5% NaOCl Ace, Turkey) for 20 minutes. Sterilized seeds were rinsed 3 times in sterile distilled pure water. A very high rate of contamination was observed when the seeds were sterilized without peeling the husks. Thereafter, the zygotic embryos were removed from the seeds with fine dissection blade very carefully and used as explant. Zygotic embryos were cultured in MS medium [12] containing 1, 2, 3, 4, 5, 6, 7, 8 mg/l 2,4-D to which 3% sucrose was added and solidified with 0.65% agar (Duchefa). Zygotic embryos were cultured using 16 hours of light (35µmol $m^{-2}s^{-1}$) and 8-hour dark photoperiod in the environmental cabinet (Aralab). The temperature of the environmental cabinet was adjusted to 24 ± 1 ℃. The MS media used in this study were sterilized in an autoclave keeping them at 1.5 atmospheric pressure and 121 ℃ for 20 minutes. The pH of the all medium was adjusted to 5.8 ± 0.1 using 1M NaOH and 1M HCl. Thirty (30) explants were used for each treatment combination and the experiments were repeated 3 times.

RESULTS AND DISCUSSION

In our study, *Triticum monococcum* ssp. monococcum zygotic embryos were used as explant sources. The zygotic embryos were cultured on MS medium containing eight different concentrations of 2,4-D (1, 2, 3, 4, 5, 6, 7, 8 mg / l). The effect of 2,4-D concentrations on embryogenic callus formation ratio and on embryogenic callus weight is shown in Table 1. Embryogenic callus induction was noted

on all 2,4-D concentrations. The maximum calli weight was induced on MS medium containing 4 mg/l 2,4-D (Figure 1), while the minimum calli weight was obtained on the MS medium containing 8 mg/l 2,4-D (Figure 1). It was seen that each increase in concentration of 2,4-D also resulted in increase in callus weight of 289.21±2.83, 310.72±1.96, 356.63±1.06, 379.47±2.35 mg on MS medium containing 1, 2, 3 and 4 mg/l 2,4-D respectively. This showed that each increase in weight was significantly higher compared to weight gained on each of the previous concentration of 2,4-D. Thereafter, each concentration in 2,4-D had inhibiting effect that showed significant reduction of 361.34±1.56, 305.19±2.53 and 276.67±1.18 and 268.21±2.13 mg callus weight on each increasing concentration of 5, 6, 7 and 8 mg/l 2,4-D respectively.

Our results show that the 2,4-D concentrations in MS medium is quite effective on induction of embryogenic callus and their weight.

The results of the study are in agreement with Ahmad et al. [2], who observed that maximum callus formation was induced in Ruta graveolens L. Using 10 µM 2,4-D. They reported that 2,4-D induced callus formation percentage positively increased or decreased depending on the concentration of 2,4-D.

Mukherjee et al. [11] evaluated the results of the NAA+ Kinetin and 2,4-D + Kinetin on callus induction of *Clerodendrum indicum*, a plant that grows in India and is used as bio remediation of heavy metals from the environment. They reported that the optimal combination for callus formation was 1 µM NAA + Kinetin 0.5 µM and 3.0 µM 2,4-D + 3.0 µM Kinetin . The finding of that study suggested that medium containing 2,4-D was inductive to callus formation. In accordance to these findings, the results of this study showed that MS medium containing different concentrations of 2,4-D induced variable percentage of callus.

Table 1. The effects of the 2,4-D concentration on callus formation percentage and callus weight.

Concentration of 2,4-D (mg/l)	Embryogenic callus induction percentage (%)	Embryogenic callus weight (mg)
1.00	%100.00	289.21±2.83d
2.00	100.00	310.72±1.96c
3.00	100.00	356.63±1.06b
4.00	100.00	379.47±2.35 a
5.00	100.00	361.34±1.56b
6.00	100.00	305.19±2.53c
7.00	100.00	276.67±1.18d
8.00	100.00	268.21±2.13e

All values shown by different small letters in a colomun are statistically different using Duncans multiple range test as $p<0.05$ level of significance

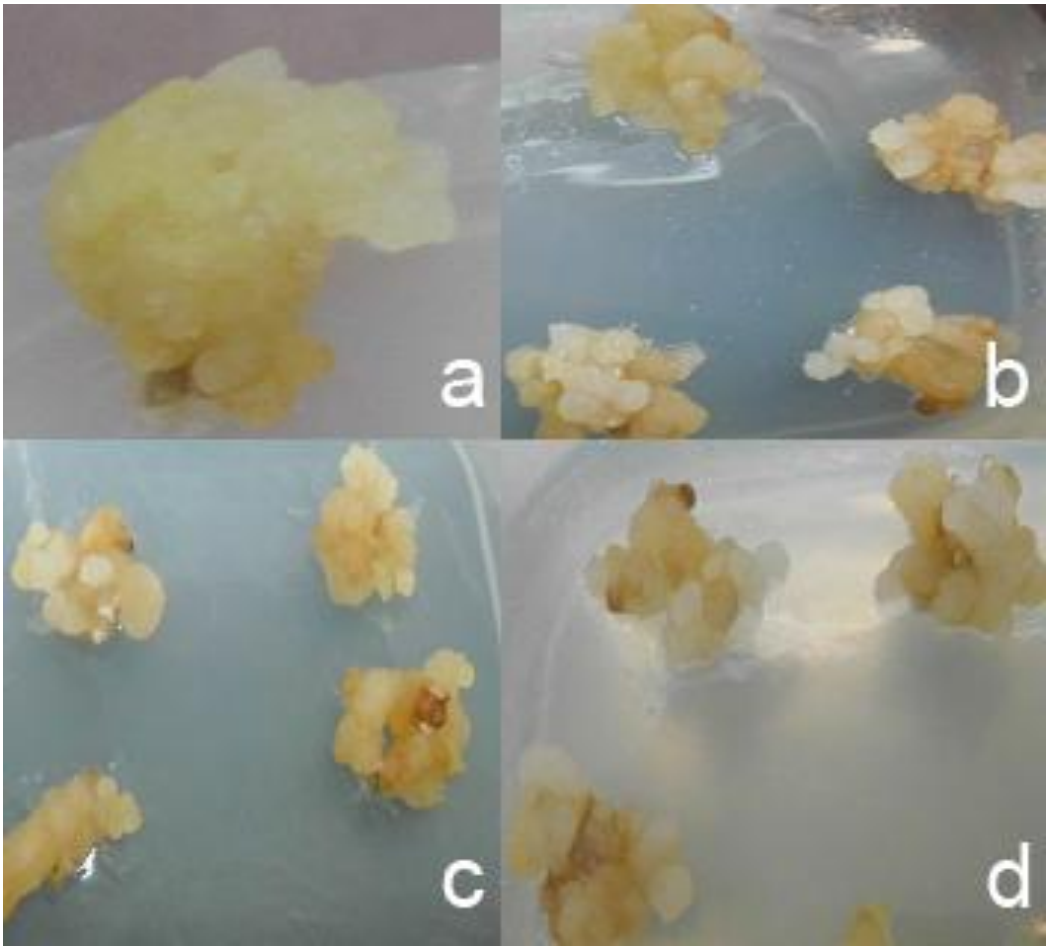

Figure 1. MS medium containing 4 mg/l 2,4-D which resulted in induction of maximum callus weight (A), Embryogenic calli growing on MS medium containing 3 mg/l 2,4-D (B), MS medium containing 8 mg/l 2,4-D which resulted in minimum callus weight (C), Embryogenic calli growing on MS medium containing 5 mg/l 2,4-D (D).

The results of this study also confirmed studies of Ozias-Akins and Vasil [13], who noted effects of different amounts of 2,4-D (0; 0.4; 1.0; 4.0 and 8.0 mg/l) in Gamborgs B5 medium and found all had inductive effect on the callus formation and development for *T. aestivum*. These findings showed that callus fresh weight per explant and the number of cells increased with rising concentrations of 2,4-D. When the 2,4-D concentration was 2 mg/l or higher the callus growth was irregular, but showed consistent increase in cell division. They also reported that 2 mg/l 2,4-D was optimum for callus formation and development. The results of this study showed that the MS medium containing 4 mg/l 2,4-D produced the heaviest calli and when the concentration of 2,4-D raised above 4 mg/l, or fell below 4 mg/l a reduction of callus weight was determined. They also showed that concentrations of 2,4-D higher than 2 mg/l had a negative impact on the amount of callus formation and development. The results support our studies. It was found that for 2,4-D concentrations below 4 mg/l were non callus inductive and maximum callus induction was noted on 5 mg/l 2,4-D. Higher 2,4-D concentrations showed a sharp inclination in callus formation rate. As seen in this study, a certain amount of the 2,4-D concentration stimulates the callus formation while higher concentrations had a negative effect on the callus production. 4 mg/l 2,4-D concentrations was most optimum concentration and use of concentrations above or below this concentration affected callus weight negatively. Other authors [1, 7, 8, 9, 14, 15, 19] suggest that callus formation is strictly explant and type of plant growth regulator/s combinations used in multiplication of monocotyledonous or dicotyledonous plants.

CONCLUDING COMMENTS

As a result, it is deduced that 2,4-D concentrations used in this study played an active role in induciton of embryogenic cali in *T. monococcum* ssp monococcum using zigotic embryos.

REFERENCES

[1] Aasim, M. Khawar, K.M.,Özcan, S. 2009. Comparison of shoot regeneration on diferent concentrations of Thidiazuron from shoot tip explant of cowpea on gelrite and agar containing medium. Notulae Botanicae Horti Agrobotanici Cluj-Napoca.37, (1), : 89-93.

[2] Ahmad, N., Faisal, M., Anis, M., Aref, I.M., 2010, In vitro callus induction and plant regeneration from leaf explants of *Ruta graveolens* L., South African Journal of Botany, 76: 597–600.

[3] De Vincenzi, M., Luchetti, R., Giovannini, C., Pogna, N.E., Saponaro, C., Galterio G., Gasbarrini, G., 1996. In vitro toxicity testing of alcohol soluble proteins from diploid wheat *Triticum monococcum* in celiac disease. Journal of Biochemical and Molecular Toxicology, 11, (6).

[4] Fasano, A., Catassi, C., 2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology, 120, 636–651.

[5] Hidalgo, A., Brandolini, A., 2008. Protein, ash, lutein and tocols distribution in einkorn (*Triticum monococcum* L. subsp. monococcum) seed fractions. Food Chemistry 107, 444–448.

[6] Hidalgo, A., Brandolini, A., Pompei, C., Piscozzi, R., 2006. Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. monococcum L.). Journal of Cereal Science, 44, 182–193.

[7] Khawar, K.M., Ozcan, S. 2002. High frequency shoot regeneration from cotyledonary node explants of different lentil (*Lens culinaris* Medik) genotypes and in vitro micrografting Biotechnology and Biotechnological Equipment. 16 (1): 12-17.

[8] Khawar, K.M.,Cocu, S.Parmaksiz, I.,Sarihan, E.O., Özcan, S. 2005. Mass proliferation of madonna lily (Lilium candidum L) under in vitro conditions Pakistan Journal of Botany.37, (2): 243-248.

[9] Khawar, K.M., Sarihan, E.O.,Sevimay, C.S., Çöçü, S.,Parmaksiz, I.,Uranbey, S., Ipek, A.,Kaya, M.D.,Sancak, C.,Özcan, S. 2005. Adventitious shoot and regeneration and micropropagation of *Plantago lanceolata* L. Periodicum Biologorum. 107(1): 113-116

[10] Krishna RB, Reddy SRR, Javangula H, Swapna D and Reddy KJ, 2012, Isolation and characterization of gymnemic acid from *Gymnema sylvestre* R. Br in the control of diabetes, J. of Life Sci. and Phar. Res.,2(1): 27-29.

[11] Mukherjee, A., Bandyopadhyaya, A., Duttaa, S. ve Basub, S., 2013, Phytoaccumulation of iron by callus tissue of *Clerodendrum indicum* (L), Chemistry and Ecology, 29 (6): 564–571

[12] Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum, 15: 473-97.

[13] Ozias-Akins P. and Vasil, I. K., 1983, Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (wheat), Protoplasma, 117: 40–44.

[14] Ozdemir, F.A., Yildirim, M.U., Pourali Kahriz, M. 2014. Efficient Micropropagation of Highly Economic, Medicinal and Ornamental Plant *Lallemantia iberica* (Bieb.) Fisch. and C. A. Mey. BioMed Research International. 2014, 476346.

[15] Parmaksiz, I., Khawar, K.M.2006. Plant regeneration by somatic embryogenesis from immature seeds of Sternbergia candida Mathew et T. Baytop, an endangered endemic plant of Turkey. Propagation of Ornamental Plants. 6(3): 128-133.

[16] Pizzuti, D., Buda, A., D'odorico, A., D'inca, R., Chiarelli, S., Curioni, A., Martines, D., 2006. Lack of intestinal mucosal toxicity of *Triticum monococcum* in celiac disease patients. Scandinavian Journal of Gastroenterology, 41, 1305-1311.

[17] Vaccino, P., Becker, H.A., Brandolini, A., Salamini, F., Kilian, B., 2009. A catalogue of Triticum monococcum genes encoding toxic and immunogenic peptides for celiac disease patients. Molecular Genetics and Genomics, 281, 289-300.

[18] Vincentini, O., Maialetti, F., Gazza, L., Silano, M., Dessi, M., De Vincenzi, M., Pogna, N.E., 2007. Environmental factors of celiac disease: Cytotoxicity of hulled wheat species *Triticum monococcum*, T. Turgidum ssp. dicoccum and T. aestivum ssp. Spelta. Journal of Gastroenterology and Hepatology, 22, 1816–1822.

[19] Yildirim, M.U. 2013.Micropropagation of *Origanum acutidens* (Hand.-Mazz.) Ietswaart using stem node explants.The Scientific World Journal. 2013, Article number 276464.

[20] Wieser, H., 2001. Comparative investigations of gluten proteins from different wheat species. III. N-terminal amino acid sequences of α-gliadins potentially toxic for coeliac patients. European Food Research and Technology, 213, 183–186.