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In vitro plant regeneration from mature embryos of wheat (*Triticum aestivum* L.)

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Abstract
The present study was conducted at Plant Breeding and Biotechnology Laboratory, Agrotechnology Discipline, Khulna University, Khulna during February 2014 to December 2014 with a view to investigate the genotypic effect of 16 Bangladeshi wheat varieties on callus formation from mature seed embryos and followed by plant regeneration. MS basal salts with 3 mgL^{-1} 2,4-D and 0.5 mgL^{-1} Kn was investigated for callus and hormone free MS media was selected for plant regeneration. Results of the experiment revealed that callus frequencies were significantly influenced with the wheat genotypes and callusing rate varied from 0 to 88%. Wheat genotype Aghrani, Bijoy, Sufi, Sonalika, Sourav, Barkot, Kolyansona, Prodip and Kheri did not respond at all to callus induction. Maximum and statistically highest callus frequency was noticed with the variety Pavon-76 followed by BARI gom 25 (68%), Akbar (64%) and Kanchan (28%) respectively. Study on morphogenesis of plated calli also showed variable results (0-61%). Whole plant regeneration ranged from 0 to 26%. Maximum regenerated plantlets obtained from the calli of the variety BARI gom 21 followed by BARI gom 25 (19%), Akbar (15%) and Pavon-76 (13%) respectively. Among the 16 wheat genotypes, variety Pavon-76 responded best in callusing whereas BARI gom 21 performed better in whole plant regeneration.

Keywords: Wheat, mature embryo, callus, plant regeneration

1. Introduction
Wheat (*Triticum aestivum*) is the world's most important grain crop belongs to the family Poaceae. In Bangladesh, wheat ranks just after rice, planted around 429,607 hecters compared 416,522 of the last year. The average yield is 3.033 metric tons per hecter which is 0.66% higher than that of previous year. In 2012-2013 fiscal year, the total wheat production has been estimated at 13, 02, 998 metric tons compared to 12, 54, 778 metric tons of the last year, which is 3.84% higher [2]. The traditional breeding methods of wheat have been practiced for centuries and still it is the main tool to breed new varieties. But some special breeding methods like mutation, haploid, somaclonal variation, genetic engineering etc. also have been gaining importance in recent years for creation new variability to combat specific stresses [3,7] . In recent years, biotechnology is emerging as one of the latest tools of agricultural research. Biotechnology involves the systematic application of biological processes for the beneficial use of mankind. One of the areas of plant biotechnology involves the delivery, integration and expression of defined genes into plant cells, which can be grown in artificial culture media to regenerate plants. Thus biotechnological approaches have the potential to complement conventional methods of breeding by reducing the time taken to produce cultivars with improved characteristics. The introduction of foreign genes encoding for resistance against pests and pathogens can reduce the degradation of the environment due to the use of hazardous biocides [10] .Many factors are responsible for the frequencies of callus induction and plant regeneration in wheat tissue culture such as culture medium composition, sources of explants, genotype and environment. Among them the genotype and nutrient composition are major sources of variation in in vitro culture [4,5] .

Callus culture through mature and immature embryo has often been applied to solve some practical problem in wheat breeding. Callus induction frequencies vary genotype to genotype [6,8] and also vary much dependent on a proper combination and concentration of plant growth hormone [11] . Massive callus induction and subsequent plant regeneration are the prerequisite for successful genetic engineering and for the induction of somaclonal variation. So understanding of the improvement of genetically improved varieties and to get maximum output from high yielding genotypes under different varietal and hormonal condition is important since research is in progress to these experiments. The present study was undertaken to observe varietal response on callus induction from mature wheat seed embryos and further proliferation.

2. Materials and Methods

2.1 Wheat variety

Sixteen Bangladeshi wheat varieties viz. Aghrani, Bijoy, Prodip, Pavon-76, Kanchan, Sufi, Barkat, Kheri, Akbar, Sonalika, Protiva, Sourav, Kolyansona, Gourav, BARI gom 21, BARI gom 25 were selected as experimental materials. The seeds were collected from Regional Agriculture Research Station (RARS), Jessore, Bangladesh.

2.2 Types of explant

Mature seed embryos were used as explants for callus induction.

2.3 Treatments:

16 wheat varieties were taken as treatment. Mature seed embryos of these varieties were cultured on MS medium supplemented with 3 mgL^{-1} 2,4-D and 0.5 mgL^{-1} Kinetin. For plant regeneration hormone free MS medium was selected.

2.4 Culture of explants and incubation

After sterilization the mature seeds were kept separately in autoclaved distilled water in culture bottle for 18 hours of incubation of seeds. This was done just for smooth isolation of embryos from seeds. Mature embryos were isolated from the imbibed seeds with the help of needle and forceps aseptically in laminar air flow. Then they were transferred onto nutrient medium. The cultures were incubated at 25±1° C in the dark for 6 weeks to allow callus formation from the somatic tissue of mature and immature embryos.

2.5 Callus induction and transfer into regeneration medium

Calli started initiating after two weeks of culture in case of mature embryos. Calli were transferred onto the test-tube containing hormone free MS medium after 6 weeks of the culture. In this condition, the cultures were kept in light (2000-3000 lux) with a temperature of 25±1° C maintaining humidity 60-70%. Calli were transferred onto the MS medium containing test-tube due to decrease of nutrient after two weeks of transference of calli onto regeneration medium. No growth regulators were added with MS medium. After one month of first sub-culture, second sub-culture was done on the same medium. Final plant transferred into soil.

2.6 Design of experiment

The experiment was laid out in the laboratory following Completely Randomized Design (CRD) with five replications. A cultured bottle containing 10 embryos constituted a replication.

2.7 Collection and analysis of data

Data were collected by counting the calli induced and plants, roots or shoots regenerated in the test tubes. After one month of inoculation of immature and mature wheat embryos, callus induction frequency was calculated. All the calli originated from a single seed and all the plants originated from a single piece of callus were considered as one.

The frequency of callus induction and plant regeneration was calculated as bellow:

$$\text{Callus induction frequency (\%)} = \frac{\text{Number of seeds produced calli}}{\text{No. of seeds plated}} \times 100$$

$$\text{Plant regeneration frequency (\%)} = \frac{\text{Number of calli regenerated into plants}}{\text{No. of calli plated}} \times 100$$

$$\text{Root regeneration frequency (\%)} = \frac{\text{Number of calli regenerated into roots}}{\text{No. of calli plated}} \times 100$$

$$\text{Shoot regeneration frequency (\%)} = \frac{\text{Number of calli regenerated into shoots}}{\text{No. of calli plated}} \times 100$$

Total morphogenesis (%) = Plant regeneration frequency (%) + Root regeneration frequency (%)

Recorded data on the frequency of callus induction were analyzed for Variance (ANOVA) with the help of statistical package program (SPSS) in computer. Differences among the means were compared by Duncan's New Multiple Range Test (DMRT).

4. Results and Discussion

4.1 Effect of wheat genotype on callus induction from mature embryo explants

For callus induction from mature embryo explants, 16 wheat varieties were tested. Among the varieties studied only seven were responded in callus induction. Rest Kheri, Sonalika, Kolyansona, Barkat, Aghrani, Sufi, Bijoy, Prodip and Sourav did not show any sign of proliferation of callus tissue. Mean frequency of callus formation was varied with different varieties (Table 1).

Table 1: Effect of wheat varieties on callus induction from mature embryos

Variety	No. of embryos inoculated	No. of embryos forming callus	Frequency of callus (%)
Aghrani	50	0	0 d
Pavon-76	50	44	88 a
Bijoy	50	0	0 d
Sufi	50	0	0 d
Kanchan	50	14	28 c
Akbar	50	32	64 b

Sonalika	50	0	0 d
Sourav	50	0	0 d
Protiva	50	20	40 c
Barkat	50	0	0 d
Kolyansona	50	0	0 d
Gourav	50	21	42 c
Prodip	50	0	0 d
Kheri	50	0	0 d
BARI gom 21	50	22	44 c
BARI gom 25	50	34	68 b
Significance	0.01		

Data followed by same alphabet(s) in a column are not significantly different according to DMRT.

Maximum number of callus (88 %) was obtained from the variety Pavon-76 which was significantly higher than others. On the other hand, the second most potential varieties were BARI gom 25 and Akbar producing 68 % and 64 % callus respectively. The lowest frequency was obtained from the variety Kanchan producing callus 28% but statistically similar to Protiva, Gourav and BARI gom 21 forming callus 40 %, 42 % and 44 % respectively.

4.2 Morphogenesis of plated calli derived from mature embryos

Calli derived from mature embryos of seven wheat varieties were sub-cultured 4 times on full strength hormone free MS medium at 15 days interval. It was found that some of the calli regenerated only into shoots without any root formation, few of the calli produced only roots but others into whole plant comprising both root and shoot. Either the calli produced only shoot, only root or regenerated into green plants, all of the cases were considered as morphogenesis. For the study of the morphogenesis, the effect of variety was investigated.

Morphogenesis is higher (61%) in the calli produced from BARI gom 25, followed by Protiva (57%), Pavon-76 (51%), Akbar (42%), Kanchan (41%) and BARI gom 21 (39%). But the calli of the variety Gourav did not respond at all in morphogenesis. In whole plant regeneration BARI gom 21 performed better (26%) than other varieties.

Table 2. Effect of wheat varieties on morphogenesis of plated calli

Variety	No. of calli inoculated	Calli responded to morphogenesis (%)			
		Root	Shoot	Whole plant	Total
Pavon-76	31	19	19	13	51
Kanchan	17	29	12	0	41
Akbar	21	9	18	15	42
Protiva	7	57	0	0	57
Gourav	8	8	0	0	0
BARI gom 21	15	13	0	26	39
BARI gom 25	26	42	0	19	61

In the present experiment, sixteen different wheat varieties were tested for callus induction and subsequent plant regeneration. Callus induction rate was variable with the various wheat genotypes. Among the varieties, Pavon-76 was found the most efficient for callusing (88 %) followed by BARI gom 25 (68%) and Akbar (64%) produced respectively. Nine out of 16 varieties did not produce any callus. Different authors reported genotype dependent callusing and plant regeneration in wheat from mature embryos in *in vitro* condition [6,8,9] which is in agreement with our findings. Results in this experiments should that both callus formation and morphogenesis were influenced by wheat genotypes. Regeneration potentiality of calli derived from different genotypes through mature embryos were tested. Among the test varieties calli of BARI gom 25 responded better in morphogenesis (61%) but calli of variety Gourav did not produce any callus.

5. Conclusion

Finally it can be concluded that among the 16 wheat genotypes, variety Pavon-76 responded best in callusing whereas BARI gom 21 performed better in whole plant regeneration.

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7. References

1. Anderson JV, Davis DG. Abiotic stress alters transcript profiles and activity of glutathione *S*-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula*. Physiol Plant, 2004; 120: 421-433.
2. BBS.2014.www.bbs.gov.bd/WebTestApplication/userfiles/Image/Agriculture/wheat 2013-14.pdf. 24th october, 2014.
3. Dolgikh YI, Osipova, ES., Solo'eva, AI, Lysenko EA, Vysotskaya, ON. 2012. Genetic variability of cultured plant tissues under normal conditions and under stress. Journal of Stress Physiology and Biochemistry 8,11.
4. Khanna HK, Raina,SK. 1998. Genotype X culture media interaction response of three indica rice cultivars. Plant Cell, Tissue and Organ Culture 52,145-153.
5. Khatun M, Ali MH, Desamero NV. 2003. Effect of genotype and culture media on callus induction and plant regeneration from mature seed scutellum culture in rice. Plant Tissue Culture 13, 99-107.
6. Mahmood H, Zaheer A, Muhammad M, Saad IM, Khurram S. 2009. Effect of sorbitol in callus induction and plant regeneration in wheat. African Journal of Biotechnology, 8, 6529-6535.
7. Micke A, Donini B, Maluszynski M. 1990. Induced mutation for crop improvement. Mutation Breed. Rev. 7, 1-41.
8. Najat H, Sripada, MU, Fatima G, Abdelwahd R, Mohamed I. Driss I. 2014. Effect of genotypes and

culture media on embryogenic callus induction and plantlet regeneration from mature embryos of durum wheat. Romanian Agricultural Research 31, 50-58.
9. Parvin E, Ezatollah F, Mostafat A. 2012. Response of bread wheat genotypes to immature embryo culture, callus induction and drought stress. Current Research Journal of Biological Sciences 4, 372-380.
10. Roco A , Pérez LM. 2001. In vitro biocontrol activity of *Trichoderma harzianum* on *Alternaria alternata* in the presence of growth regulators. Electronic Journal of Biotechnology 4,1-6.
11. Shafquat Y, Imtiaz AK, Abdullah K, Nighat S, Ghulam SN, Mohammad AA. 2009. In vitro plant regeneration in bread wheat (*Triticum aestivum* L.). Pak. J. Bot. 41, 2869-2876.