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Effect of callus induction media and phytohormones on regeneration of shoots in spring wheat

M.R. Khatun, M.M. Howlader[1], M.M. Hossain[1], R.Dey[2] and Y.Masuma[3]
Agricultural Training Institute, Daulatpur, Khulna, [1]On -Farm Research Division, BARI, Khulna, [2]Department of Agriculture Extension, Savar, Dhaka, [3]Planning and Evaluation Wing, Department of Agriculture Extension, Dhaka.

Abstract: The experiment was conducted in the tissue culture laboratory and glass house of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh during November 2003 to June 2004 to evaluate the relative efficiency of different culture media for callus initiation and subsequent shoot regeneration as well as screening of wheat varieties for their good regeneration potentiality. For this study five varieties of spring wheat viz. Gaurav, Kalyansona, Balaka, Aghrani, Soughat; five callus induction medium viz. MS medium (control), MS medium+2,4 D at 2.0 mg/L, MS medium+ 2,4 D at 4.0 mg/L, MS medium + picloram at 1.0 mg/L, MS medium+ picloram at 2.0 mg/L and six doses of different phytohormones viz. MS medium (control), MS medium + BAP at 0.5 mg/L, MS medium+ BAP at 1.0 mg/L, MS medium+ kinetin at 0.5 mg/L, MS medium + kinetin at 1.0 mg/L and MS medium + BAP at 0.5 mg/L+ kinetin at 0.5 mg/L were taken. The variety Aghrani found to be the best for callus initiation. It required minimum days (3.2) for initiation of callus. The largest size of callus was observed in Soughat (4.16 mm). Callusing was found delay in Gaurav (3.6 days). The large callus (4.31) was obtained from Soughat in MS medium supplemented picloram at 2.0 mg/L.Variety Balaka took minimum days (23.6) for initiation of shoot, while the maximum days were required in Gaurav (24.9). Supplementation of BAP 0.5 mg/L in MS medium enhanced early shoot initiation (23.3) and maximum days was required in control (26.5). Variety Ahgrani produced the highest number (2.7) of shoots per callus and the lowest was found in Kalyansona and Soughat (2.24). The variety Aghrani gave the highest number of shoots per callus that was observed in MS medium supplemented with kinetin at 1.0mg/L (2.73) which was statistically similar with Aghrani × BAP 1.0 mg/L (2.66) and Soughat × MS+BAP 0.5mg/L+ kinetine 0.5mg/L(2.58)
Key words: Induction Media, Phytohormones, Regeneration, Spring Wheat.

Introduction

Two-third of the world population consumes wheat (*Triticum aestivum* L.) as a major source of calorie. Cultivation of wheat has been popular to the farmers of Bangladesh after the worldwide successful campaign of green revolution in the mid sixties. The Wheat Research Center (WRC) of Bangladesh Agricultural Research Institute (BARI) released 21 wheat varieties which yield hardly approached to 3 tons per hectare in farmers field. But in some wheat growing countries it is about 8 tons per hectare (FAO, 1999). So, it is essential to increase yield potentiality through improving yield contributing characters. To improve agronomic characters of wheat, conventional breeding methods were tried but these were not so successful due to narrow genetic base. Moreover conventional technique takes more time for crop improvement. Now a days plant tissue culture techniques have been developed as a modern and worldwide accepted concept. For the development of modern crop varieties, tissue culture technique is now widely used in many plant breeding programmes. Tissue culture techniques along with plant molecular biology and gene manipulation technique show promising to increase the efficiency of conventional breeding methods (Shenoy and Vasil, 1992). Callus initiation and subsequent shoot regeneration is somehow dependent on the type of explants, culture media, suitable hormones, genotypes and effect of temperature and light. Immature embryos are the most efficient tissue source for regeneration of wheat plants in large numbers (Cooper *et al.*, 1986). Both callus initiation and shoot regeneration from explants require the presence of appropriate combinations and concentration of growth regulators in culture media.The present research work was, therefore, undertaken to study the relative efficiency of different culture media for callus initiation and subsequent shoot regeneration,to screen five wheat varieties for their good regeneration potentiality, and to standardize an in vitro regeneration protocol for the genetic improvement of wheat varieties using biotechnological approach.

Materials and Methods

The experiment was conducted in the tissue culture laboratory and glass house of the Department of Genetics and plant Breeding, Bangladesh Agricultural University, Mymensingh, during the period of November 2003 to June 2004. Five varieties of spring wheat viz. Gaurav, Kalyansona, Balaka, Aghrani and Soughat were taken in the present study. Five medium viz. MS medium (control), MS medium+2,4 D at 2.0 mg/L, MS medium+2,4 D at 4.0 mg/L, MS medium + picloram at 1.0 mg/L and MS medium+ picloram at 2.0 mg/L for callus induction and six medium viz. MS medium (control), MS medium+ BAP at 0.5 mg/L, MS medium+ BAP at 1.0 mg/L, MS medium+ kinetin at 0.5 mg/L, MS medium+ kinetin at 1.0 mg/L and MS medium +BAP at 0.5 mg/L+ kinetin at 0.5 mg/L for regeneration were taken as treatment. MS medium was used as a basal medium for both induction of callus and regeneration of shoots from the callus. The preparation of MS medium usually consists of organic and inorganic salts, irons and a carbon source (Murashige and Skoog, 1962). As different ingredients were required in different concentrations, separate stock solutions for macro-nutrients, micro-nutrients, vitamins and growth hormones were prepared. Stock solution A (macro-nutrients), stock solution B (micro-nutrients), stock solution C (vitamins) and stock solution of hormone for callus induction and shoot regeneration media were autoclaved with 1.16 Kg/cm^2 pressure at 12 PC for 30 minutes. Glass wares were rapped with aluminum foil, vials were capped with plastic cap and then were sterilized in an autoclave at temperature of 121^0 C for 30 minutes at 1.16 kg/cm^2 pressure. Fourteen days immature caryopsis were dipped in 70% ethyl alcohol for five minutes and shaking followed by washing with sterile distilled water and soaking for 15 minutes in 30% chlorax. Then the immature embryos were aseptically excised from caryopsis and placed with the scutellum upwards on a solid agar medium containing 2,4-D and picloram in sterile Petridish. Ten immature embryos were cultured per pertidish and the petridishes were sealed with parafilm.

The cultured explants were incubated in darkness with controlled temperature (25±1^{0}C) for 21 days. Callus was initiated three days after inoculation and after 3 weeks inoculated explants were transferred onto fresh regeneration media for root and shoot development. When the plants produced sufficient roots, it transplanted to small plastic pots containing potting mixture. Pots were covered with moist polyethylene bag to prevent desiccation. After two to three days the polyethylene bags were gradually perforated to expose the plants to natural environment. The polyethylene bags were completely removed after 7 days. Finally after 10-15 days, the plants were transferred to the field condition where they developed into mature plants. Data on days to callus initiation, callus size, days to shoot initiation and number of shoots per callus were recorded and data were analyzed statistically. Means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Varietal response to callus initiation: Different varieties showed the significant variation in callus initiation. Performance among the varieties for callus initiation and its size are presented in the Table 1. The range of days required for callus initiation varied from 3.2 to 3.6 days. The variety Gaurav took the maximum time (3.6 days) while the variety Aghrani took the minimum time (3.2 days) for callus initiation, statistically similar result were recorded with Kalayansona, Balaka and soughat. Maddock *et al.* (1983) reported that the capability for callus initiation of wheat dependent on the genotypes.

The range of callus size varied from 3.76 mm to 4.16 mm. The variety Soughat produced the largest size (4.16 mm) of callus which was statistically similar with the callus of Gourav (4.01mm) and the lowest callus size (3.76mm) was produced by the Balaka which was statistically similar with Kalyansona and Aghrani.

Table 1. Performance of the wheat varieties for callus initiation

Variety	Days to callus initiation	Mean Callus size (mm)
Gaurav	3.6	4.01
Kalyansona	3.4	3.93
Balaka	3.4	3.76
Aghrani	3.2	3.89
Soughat	3.3	4.16
LSD (0.01)	0.21	0.22
CV(%)	5.68	5.05

Callus initiation due to induction media: Minimum days (3.2) for callus initiation was observed in MS+ picloram 1.0 mg/L and MS+ picloram 2.0 mg/L which was statistically similar with MS+ 2,4-D 4.0 mg/L. Maximum days (3.6) required for callus initiation when induction media was prepared with 2,4-D 2.0 mg/L.

MS medium supplemented 2.0 mg/L of picloram gave the highest callus size (4.16 mm) and it was statistically identical with MS+ picloriam 1.0mg/L. while the minimum callus size (3.80 mm) was recorded in MS medium supplemented with 2.0 mg/L of 2,4-D (Table 2). Kosulina (1995) stated that increase in picloram gave good callus size.

Table 2. Effect of induction media on callus initiation

Induction media	Days to callus initiation	Callas size (mean) (mm)
MS+2,4-D 2.0 mg/L	3.6	3.80
MS+2,4-D 4.0 mg/L	3.3	3.82
MS+ Picloram 1.0 mg/L	3.2	4.04
MS+ Picloram 2.0 mg/L	3.2	4.16
MS(control)	No callus	No callus
LSD (0.01)	0.18	0.19
CV (%)	5.68	5.05

Table 3. Interaction between variety × induction media on callus initiation

Variety	MS+Hormone	Days to callus initiation	Callus size (mm)
Gaurav	MS+ 2,4-D 2.0 mg/L	3.9	4.10
	MS+ 2,4-D 4.0 mg/L	3.1	3.88
	MS+ Picloram 1.0 mg/L.	3.8	4.04
	MS+ Picloram 2.0 mg/L	3.6	4.03
Kalyansona	MS+ 2,4-D 2.0 mg/L	3.5	3.64
	MS+ 2,4-D 4.0 mg/L	3.6	3.74
	MS+ Picloram I.0 mg/L	3.2	4.16
	MS+ Picloram 2.0 mg/L	3.1	4.19
Balaka	MS+ 2,4-D 2.0 mg/L	3.5	3.34
	MS+ 2,4-D 4.0 mg/L	3.3	3.77
	MS+ Picloram I.0 mg/L	3.4	3.90
	MS+ Picloram 2.0 mg/L	3.2	4.04
Aghrani	MS+ 2,4-D 2.0 mg/L	3.6	3.42
	MS+ 2,4-D 4.0 mg/L	3.1	4.0
	MS+ Picloram 1.0 mg/L	3. 0 L	3.94
	MS+ Picloram 2.0 mg/L	3. 0	4.23
Soughat	MS+ 2,4-D 2.0 mg/L	3.5	4.07
	MS+ 2,4-D 4.0 mg/L	3.4	4.13
	MS+Picloram1.0mg/L	3.2	4.14
	MS+Picloram2.0mg/L	3 .2	4.31
LSD (0.01)		0.42	0.44
CV (%)		5.68	5.05

Interaction between variety and induction media: Early callusing was found in the interaction of MS + picloram 2.0 mg/L and MS + picloram 1.0 mg/L with Aghrani (3.0 days). The maximum time (3.9days) required for callus initiation in MS+2.0 mg/L of 2.4-D×Gaurav (Table 3). Agarwal *et al.* (1995) observed highly significant differences between genotype and culture media as well as genotypes × medium interaction for callus formation.Size of callus was observed highest(4.31mm) in MS+2.0 mg/L picloram with Soughat which were statistically similar with MS+2.O mg/L of picloram × Agrani (4.23 mm), MS+2.0mg/L of picloram × Kalyansona (4.19 mm) and MS+ 1.0 mg/L of' picloram × Kalyansona (4.16 mm). The lowest callus size was found from MS+2,4-D 2.0 mg/L with Balaka.

Table 4. Variation among the varieties for shoots regeneration

Variety	Days to shoot initiation	No. of shoot/callus
Gaurav	24.9	2.34
Kalyansona	24.00	2.24
Balaka	23.60	2.29
Aghrani	24.10	2.70
Soughat	24.4	2.24
LSD (0.01)	1.00	0.084
CV (%)	4.66	5.79

Generation of shoots

In case of variety: In regeneration media, the days for shoot initiation range from 23.6 days in Balaka to 24.9 days in Soughat which were very close to each other. Aghrani produced the highest number of shoots (2.70) and the lowest number of shoots recorded from Soughat and Kalyansona (2.24) which was statistically similar with Balaka (2.29) (Table 4).

In case of regeneration media: Among the six treatments supplementation of BAP or kinetin in different doses enhanced early shoot initiation as compared to control. Wang *et al.* (2004) reported that adding of BAP into regeneration media shortened the time of shoot emergence from callus (Table 5).

The highest number of shoots (2.39) per callus was found in BAP 1.0 mg/L and Kinetin 1.0 mg/L, the lowest number of shoots per callus was recorded from control (2.06)

Interaction between variety and regeneration media: Interaction of the variety with different regeneration media showed range of days to shoot initiation from 22.4 days in Balaka in 0.5 mg/L of BAP to 27.4 days in Gourav in control. The maximum (27.4 days) time required for Gaurav × MS control and the minimum time recorded from Balaka × BAP 0.5mg/L (Table 6).

The highest number of shoots (2.73) per callus was observed in Agrani × kinetin 1.0 mg/L (2.73) and it was statistically similar with Aghrani × BAP 1.0 mg/L (2.66) and Soughat × BAP 0.5 mg/L+kinetin 0.5 mg/L (2.58). The lowest number of shoots per callus was recorded from Soughat in control (1.92).

From the study on callus formation and regeneration of shoots of spring wheat, it could be concluded that: (i). The variety Gaurav took the highest time and Aghrani took the lowest time for callus initiation. In case of induction media, early callusing was observed in MS + Picloram 1.0 mg/L and MS+ Picloram 2.0 mg/L. Maximum days for callus initiation was found in MS+2,4 D 2.0 mg/L.

Table 5. Effect of different regeneration media on shoots regeneration

Regeneration media	Days to shoot initiation (Mean)	No. of shoots/callus
MS (control)	26.5	2.06
MS +BAP 0.5 mg/L	23.3	2.35
MS +BAP 1.0 mg/L	23.8	2.39
MS+ Kinetin 0.5mg/L	23.7	2.29
MS+ Kinetin 1.0 mg/L	23.8	2.39
MS +BAP 0.5 mg/L + Kinetin 0.5 mg/L	23.7	2.37
LSD (0.01)	1.096	0.092
CV (%)	4.66	5.79

(ii). Variety Soughat produced the largest callus and Balaka produced the smallest sized callus. Larger size callus was observed in MS + Picloram 2.0 mg/L and small size callus was obtained from MS+2,4-D 2.0 mg/L. (iii). Balaka produced shoots in shorter time and Gaurav took maximum time. In case of regeneration media, minimum time for shoot initiation was found in MS+BAP 0.5 mg/L and the maximum time recorded in control (MS media). (iv). The highest number of shoots was recorded from Aghrani and the lowest number from Kalyansina and Soughat. Maximum number of shoots was observed in MS+BAP 1.0 mg/L and MS+ Kinetin 1.0 mg/L while minimum number of shoots was recorded from control. (v). In case of interaction Aghrani × Piclorium 1.0 mg/L and 2.0 mg/L performed better. Because it took minimum time for callus initiation. Soughat × Piclorium 2.0 mg/L produced the largest callus and larger callus found from Aghrani in the same media. Balaka × BAP 0.5 mg/L took shorter time for shoot initiation. But Aghrani × BAP 1.0 % also took minimum time for callus initiation and produced the higher number of shoots per callus simultaneously maximum number of shoots per callus was observed in Aghrani × Kinetin 1.0 mg/L.

Table 6. Interaction between variety × regeneration media on shoots regeneration

Variety	Treatment	Days to shoot initiation	Number of shoot/callus
Gaurav	MS (control)	27.4	1.99
	MS + BAP 0.5 mg/L	23.3	2.39
	MS + BAP 1.0 mg/L	22.8	2.43
	MS + Kinetin 0.5 mg/L.	24.9	2.35
	MS+ Kinetin 1.0 mg/L	25.0	2.48
	MS+ BAP 0.5 mg/L+ Kinetin 0.5mg/L	23.0	2.29
Kalyansona	MS (control)	25.2	2.01
	MS +BAP 0.5 mg/L	24.3	2.06
	MS +BAP 1.0 mg/L	23.1	2.45
	MS+ Kinetin 0.5 mg/L	23.5	2.33
	MS+ Kinetin 1.0 mg/L	24.8	2.23
	MS+ BAP 0.5 mg/L+ Kinetin 0.5 mg/L	23.0	2.37
Balaka	MS (control)	26.7	2.03
	MS +BAP 0.5 mg/L	22.4	2.48
	MS +BAP 1.0 mg/L	23.1	2.38
	MS+ Kinetin 0.5 mg/L	22.6	2.48
	MS+ Kinetin 1.0 mg/L	23.71	2.01
	MS+ BAP 0.5 mg/L+ Kinetin 0.5 mg/L	22.9	2.39
Aghrani	MS (control)	26.7	2.38
	MS +1 BAP 0.5 mg/I.	22.9	2.51
	MS +BAP 1.0 mg/L	23.8	2.66
	MS+Kinetin 0.5 mg/L	24.6	2.20
	MS+ Kinetin 1.0 mg/L	23.1	2.73
	MS+ BAP 0.5 mg/L+ Kinetin 0.5mg/L	23.3	2.20
Soughat	MS (control)	26.7	1.92
	MS +1 BAP 0.5 mg/L.	23.6	2.33
	MS +BAP 1.0 mg/L	26.4	2.33
	MS+1-Kinetin 0.5 mg/L	23.0	2.22
	MS+ Kinetin 1.0 mg/L	22.6	2.04
	MS+ BAP 0.5 mg/L+ Kinetin 0.5mg/L	26.8	2.58
LSD (0.01)		2.45	0.206
CV (%)		4.66	5.79

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