IN VITRO REGENERATION FROM MATURE EMBRYOS IN SPRING WHEAT

M.M. RAHMAN[1], A.K.M. SHAMSUDDIN[2] AND U. ASAD[3]

[1] Scientific Officer (Plant Breeding), Pulses Research Centre, Regional Agricultural Research Station, BARI, Ishurdi, Pabna, [2] Professor, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, [3]Scientific Officer (Plant Breeding), Oilseed Research Centre, Regional Agricultural Research Station, BARI, Ishurdi, Pabna, Bangladesh

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ABSTRACT

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The experiment was carried in laboratory of the Department of Genetics and Plant Breeding, BAU, Mymensingh during 2006. *In vitro* regeneration in wheat plants was carried out from mature embryos in five genotypes of spring wheat including two doubled haploids. Three concentrations of 2, 4-D were used for induction of callus. Maximum number of callus were produced on MS medium supplemented with 6.0 mg/L of 2, 4-D. But comparatively larger calli were produced on MS medium supplemented with 4.0 mg/L of 2, 4-D. Only two doubled haploid (DH-2 and DH-10) responded to regeneration. The genotype DH-10 performed better for callusing. Maximum numbers of plants were regenerated on the MS medium supplemented with 1.0 mg/L of kinetin which was followed by 2.0 mg/L of kinetin in the medium. Both the lines had similar regeneration capacity. Early shoot regeneration was observed on MS medium supplemented with 200.0 mg/L casein hydrolysate.

Keywords: Doubled haploid, mature embryo, regeneration, *in vitro*, wheat

INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the leading cereals in the world. About two thirds of the world populations live on wheat grain. In Bangladesh it ranks second next to rice (Razzaque and Hossain, 1991). Moreover, tissue culture technique provide unique possibilities for overcoming the barriers of interspecific cross, asexual gene introgression, period of dormancy etc. has also facilitated rapid development of new varieties. Tissue culture technique also offers creation of variation through somaclonal and gametoclonal variations. These variations could be exploited for crop improvement program. Therefore, plant regeneration from callus cultures could provide useful germplasm for plant breeding program. The yield and quality of wheat have been gradually improved during the past several decades by traditional breeding methods. These methods have some limitations such as long time required and rather limited gene pool available for wheat breeders (Malik *et al.*, 2003). To circumvent such problems, application of biotechnological techniques has been advocated. For such purpose, a group of activities was focused on *in vitro* culture and regeneration and haploid breeding as a tool of cereal breeding in the recent years. It is also well known that the genetic engineering of cereals currently depends on the use of tissue culture and plant regeneration techniques (Mendoza and Kaeppler, 2002).

In vitro regeneration of wheat is possible from different explants such as mature and immature embryos, seeds, endosperm, leaves, shoot bases and root tips (Sarker and Biswas, 2002). Among them the immature embryo was reported as the best for callus induction and shoot regeneration (Sarker and Biswas 2002, Arzani and Mirodjagh 1999; Hou *et al.*, 1997). But availability of immature embryo is limited by wheat growing season or requires expensive and sophisticated growth chambers. On the other hand mature seeds of wheat are readily available throughout the year, hence can be used for plant regeneration in any convenient time. High frequency of callus induction is also reported through mature embryo culture in wheat (Ozagen *et al.*, 1998). Establishing an efficient tissue culture technique is difficult in monocotyledonous species particularly in Gramineae family (Sears and Deckard, 1982). As a member of the family, wheat is also a recalcitrant crop that limits the utilization of tissue culture technique for crop improvement (Vasil and Vasil, 1986). If a suitable protocol for plant regeneration from mature embryogenic callus is available, research can be carried out on wheat transformation throughout the year. With this in view, the present study was conducted to develop a protocol for regeneration of plants from mature embryos was attempted in five genotypes of wheat including two doubled haploids.

MATERIALS AND METHODS

The experiment was carried in the green house and tissue culture laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh during 2006. Mature wheat (*Triticum aestivum* L.) embryos of five genotypes including two doubled haploid (DH) lines were used as explants for callus induction and subsequent plant regeneration. The genotypes were Sourav, Gourab, Shatabdi, DH-2 and DH-10. Mature seeds were soaked in petridish on a moistened filter paper for 2 hours before embryo dissection, so that the embryos can swell and become distinctly visible. The soaked seeds were sterilized by treating them

with 70% ethanol for 15 minutes followed by washing with distilled water. This was followed by treating for 5 min in 30% chlorox (containing 4.7% chlorine). During sterilization the seeds were occasionally agitated. Finally the seeds were washed 5 times with sterile distilled water for removing the sterilant. Then the mature embryos were aseptically excised from the caryopsis and placed on callus induction medium in sterile petridish keeping the scutellum side up. Ten mature embryos were placed in each petridish and were sealed with parafilm (Figure 1a). The callus induction media contained agar solidified MS media (Murashige and Skoog, 1962) supplemented with three different doses of 2,4-D (4.0 mg/L, 5.0 mg/L, 6.0 mg/L, respectively). The explants were incubated in dark under 25 $\pm 1^0$C for 3-5 days. When callus initiation began the petridish were transferred to light with 16 hours photoperiod under the same temperature regime. The cultures were cheeked daily to note the initiation and the development of calli. After 30 days of inoculation, the developed calli were transferred to regeneration media (Figure 1b). The regeneration media contained agar solidified MS media supplemented with different combinations and concentrations of growth regulators (MS as control, MS+ 1.0 mg/L of BAP, MS+ 2.0 mg/L of BAP, MS + 1.0 mg/L of kinetin, MS+ 2.0 mg/L of kinetin, MS + 100.0 mg/L of Casein hydrolysate and MS + 200.0 mg/L of Casein hydrolysate). All the explants, calli cultures were kept at 25±1°C under 3000 Lux light intensity and 16 hours photoperiod. The data were recorded for days to callus induction, number of callus initiated, callus size (mm), days to plant regeneration and number of plants regenerated. Data were estimated as percentage and the mean values in percent were used for analysis of data. The percent of callus induction and regeneration were estimated on the basis of the number of embryo and calli, respectively.

RESULT AND DISCUSSION

The effects of three concentrations of 2, 4-D supplemented in MS medium to induce callus from the mature embryos were evaluated. Medium supplemented with 6.0 mg/L of 2, 4-D produced maximum calli (10.08%), while medium supplemented with lower concentrations of 2,4-D produced larger size (4.40 mm) calli (Table 1). Among the genotypes, DH-10 produced maximum calli (9.83%), whereas days to callus induction and callus size were similar for all genotypes. Sarkar and Biswas (2002) reported that the MS medium supplemented with 6.0 mg/L of 2, 4-D showed the best response for callus induction from mature wheat embryos. Yasmin *et al*. (2001) observed that the mature embryos failed to initiate any type of calli at low concentrations of 2, 4-D resulting only in initial swelling. Tomar and Punia (2003) reported that increase of 2, 4-D concentration in culture media produced good callus from mature embryo of wheat. In the present study, it was observed that maximum number of small size callus were produced in high concentration of 2, 4-D but took more time for callus initiation.

Out of the five genotypes studied calli of Sourav, Gourab and Satabdi became greenish but did not regenerate (Figure 1c). Only two doubled haploid lines (DH-2 and DH-10) produced plants (Figure 1d). Effects of different combinations and concentrations of growth regulators on the regeneration of plants from these two lines of wheat are presented in the Table 2. Highest percentage of regeneration (13.63%) occurred on MS medium supplemented with 1.00 mg/L of kinetin followed by the medium with 200.00 mg/L casein hydrolysate (9.5%). On the other hand, the control treatment produced minimum percentage of plants (3.65%). From the mean value of regeneration it appears that both the genotypes had similar regeneration capacity. Longer duration (31 days) was needed for shoot initiation on the control. Plant regeneration was quick (22 days) on the treatment medium supplemented with 200 mg/L of casein hydrolysate. Malik *et al*. (2003) obtained high frequency of plant regeneration of wheat on MS medium supplemented with 0.5+0.1 mg of BAP+IAA/L. Shah *et al*. (2003) studied *in vitro* regeneration of wheat and they observed that regeneration was the highest on MS medium supplemented with 4.0 mg/L of BAP alone or 2.0 mg/L of BAP in combination with 1.0 mg /L of IAA. Sarkar and Biswas (2002) obtained maximum shoot regeneration on MS medium supplemented with 0.5 mg/L of BAP + 0.5 mg/L of kinetin. From the present study, it could be concluded that good and rapid shoot regeneration was observed on medium containing 200.0 mg/L casein hydrolysate. On the other hand higher number of plant regeneration was observed on media supplement with kinetin although it took longer time.

Table 1. Effect of different concentrations of 2, 4-D on callus induction of wheat from mature embryo

Variety	Media	Callus induction		Days to callus induction		Callus size (diameter, mm)	
		Callus (%)	Mean (%)	Days	Mean	Size	Mean
Sourav	MS+4 mg/L 2, 4-D	5.74	5.69	6	6	4.35	4.10
	MS+5 mg/L 2, 4-D	6.44		5		4.00	
	MS+6 mg/L 2, 4-D	4.90		7		3.90	
Gourab	MS+4 mg/L 2, 4-D	4.76	2.85	5	5.67	4.00	4.13
	MS+5 mg/L 2, 4-D	2.10		5		3.80	
	MS+6 mg/L 2, 4-D	1.68		7		4.60	
Satabdi	MS+4 mg/L 2, 4-D	6.44	7.10	6	5.3	4.20	4.00
	MS+5 mg/L 2, 4-D	6.44		5		4.50	
	MS+6 mg/L 2, 4-D	8.40		5		3.50	
DH-2	MS+4 mg/L 2, 4-D	8.54	8.10	4	5.3	4.40	4.23
	MS+5 mg/L 2, 4-D	6.44		5		4.40	
	MS+6 mg/L 2, 4-D	9.24		7		3.90	
DH-10	MS+4 mg/L 2, 4-D	8.26	9.83	4	5	4.35	4.21
	MS+5 mg/L 2, 4-D	9.80		5		4.30	
	MS+6 mg/L 2, 4-D	10.08		6		4.00	

Table 2. Effects of different concentration and combination of growth regulators on regeneration of wheat

Variety	Media	Plants regeneration		Days to regeneration
		Regeneration (%)	Mean (%)	
DH-2	MS	4.76	7.14	31
	MS + 1 mg/L BAP	6.35		26
	MS + 2 mg/L BAP	6.35		24
	MS +1 mg/L kinetin	7.93		27
	MS + 2 mg/L kinetin	6.35		25
	MS + 100 mg/L casein hydrolysate	8.75		23
	MS + 200 mg/L casein hydrolysate	9.5		22
DH-10	MS	3.65	7.00	30
	MS + 1 mg/L BAP	6.36		25
	MS + 2 mg/L BAP	5.45		25
	MS +1 mg/L kinetin	13.63		27
	MS + 2 mg/L kinetin	9.09		27
	MS + 100 mg/L casein hydrolysate	5.45		23
	MS + 200 mg/L casein hydrolysate	6.36		23

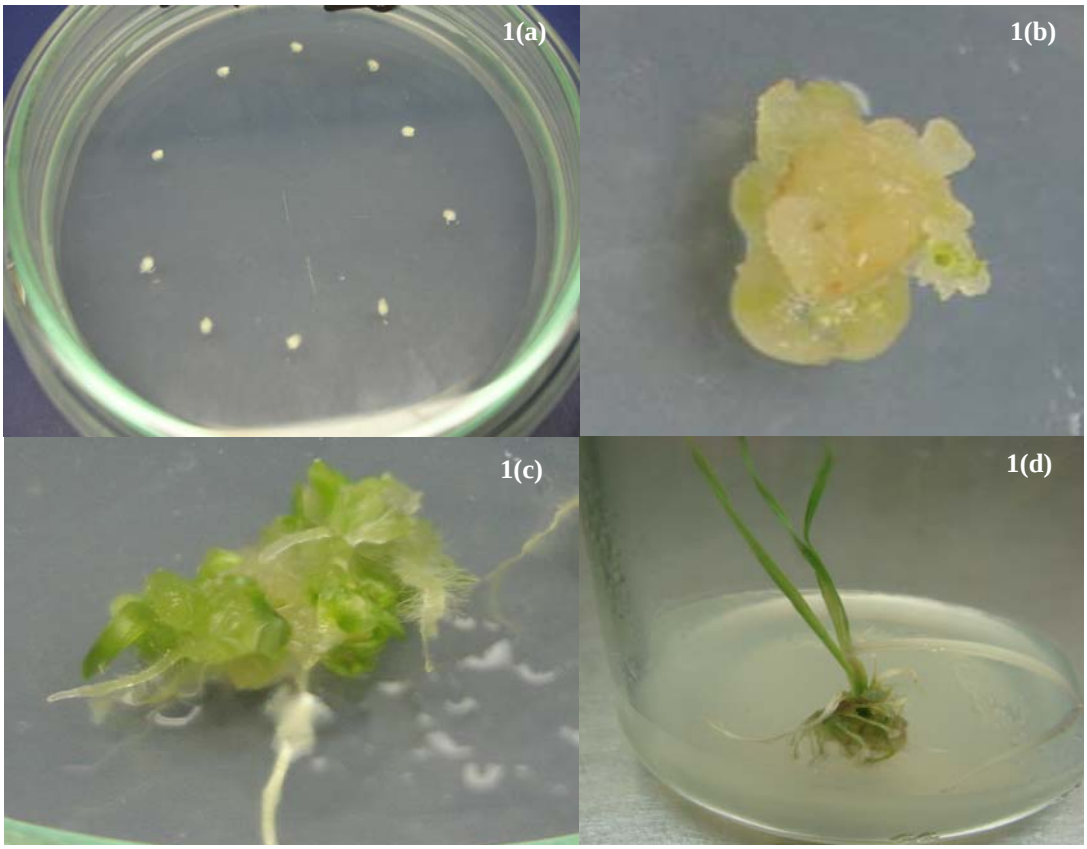

Figure 1. Different stages of *in vitro* callus induction and plant regeneration from mature embryos of wheat. (a) mature embryos on MS medium supplemented with 2.0 mg/L of 2,4-D. (b) 30 days old callus. (c) greenish callus on MS medium supplemented with 100.0 mg/L of casein hydrolysate and (d) regenerated plant from callus.

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