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EFFECT OF DIFFERENT GENOTYPES ON *IN-VITRO* CULTURE OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Wheat is notorious for callus induction, which is a major hindrance in direct gene transfer and consequently for genetic improvement program. In order to provide a successful platform for gene transformation, good callus quantity and quality is important. I was evaluated Tissue culture responses of mature embryo of ten different genotypes of wheat, which were grown in Bihar. In *in vitro* condition their responses mainly depends on their genetic makeup. This information can be exploited in crop improvement through biotechnological approaches.

Introduction

One of the important steps in the application of biotechnology for crop improvement is the successful plant regeneration from cell, organ, and tissue. Tissue culture technique provide unique possibilities for overcoming the barriers of interspecific cross, asexual gene introgression, period of dormancy etc. and 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.

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 *invitro* studies in any convenient time. High frequency of callus induction is also reported through mature embryo culture in wheat (Ozagen *et al.*, 1998). *In vitro* responses of wheat are mainly depended upon its genotype (N. Mitic *et al*., 2004). If a suitable genotype of wheat which gives better response in tissue culture is known then it's become easy to develop a new variety, and research can be carried out on wheat transformation throughout the year. With this in view, the present study was conducted to take invitro responses of different genotype of wheat grown in Bihar region.

Material and Methods

The experiment was carried out in the tissue culture laboratory, Faculty of Basic Sciences and Humanities, Rajendra Agricultural University, Pusa, Bihar during 2011. Ten different local genotype of winter wheat (*T. aestivum L.*) namely PBW-343, HD-2733, EUGANDA, KAUZ/AA/KAUZ, K-0583, PUSA GOLD, VL-914, SONALIKA, SWASN3101, C306 was used as source of mature embryos. Seeds were washed with distilled water then kept in twin 20 solution for 20-22 min followed by washing with distilled water. Surface disinfection was done with 0.1% $HgCl_2$ solution (w/v) for 3-5 min followed by several washes with sterilized distilled water. Finally seeds were kept in sterilized distilled water in closed bottle for overnight for 12-15hr. Embryo were excised inside the laminar

air flow cabinet and inoculated into MS medium supplement with various combinations and concentrations of phytohormones. All media contain 3% sucrose and 0.8% agar with pH 5.8 which was adjusted before autoclaving. For this experiment, three replication per treatment were taken and per treatment 12 tubes were used. The callus induction media MS1contain agar solidify MS media (Murashige and Skoog, 1962) supplemented with 2,4-D (4mg/L). The shoot (MS1) and root (MS3) induction media supplemented with kinetin (0.12mg/L) + BAP (0.12mg/L) and kinetin (0.5mg/L) +BAP (0.1mg/L) and 2,4-D (0.3mg/L)respectively. All the explants, calli cultures were kept at 25±1°C under 3000 Lux light intensity and 16 hours photoperiod. The responses were recorded for their frequency, day of culture, and magnitude. Responses were estimated as percentage. The percent of callus induction and regeneration were estimated on the basis of the number of embryo and calli, respectively.

Results and discussion

All three media gave the average to good response for callus induction. The highest callus frequency 92.28 percent was observed in EUGANDA on MS2 medium. On the other hand KAUZ/AA/KAUZ showed the minimum callus frequency 20.82 on MS1 medium. The genotypeHD2733 was statistically at par with PUSA GOLD on MS1 media, whereas PUSA GOLD, SAWSN 3101, HD2733, C306, K0583 and PBW343 were statistically at par with EUGANDA on MS2 media.

The only MS2 medium supplemented with KIN+BAP $0.12mgL^{-1}$+$0.12mgL^{-1}$ gave Caulogenesis responses among three media. The SAWSN3101 genotype gave highest frequency 59.25 percent of shoot induction. Whereas minimum frequency of 31.02 percent Caulogenesis was observed in VL914 genotype the PUSA GOLD genotype was statistically at par with VL914.

Two medium showed Rhizogenesis among them MS3 gave better root formation in HD2733 genotype, showed significantly highest frequency 68.01 percent. The genotypes, C306 and PUSA GOLD were statistically at par with HD2733 genotype. In MS2 two genotypes PBW343 and KAUZ/AA/KAUZ, did not showed any root formation.

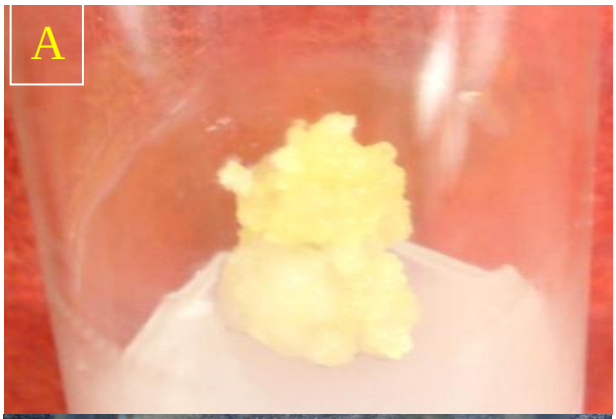
High concentration of 2, 4-D in culture media produced good callus from mature embryo of wheat Tomar and Punia (2003). The responses of ten different genotypes of wheat in MS medium to induce callus from mature embryos were evaluated. KAUZ/AA/KAUZ, EUGANDA, SONALIKA and K-0583 produce maximum calli. VL-914, PBW-343, HD-2733, PUSA GOLD produce less calli and rest C306, SWASN3101 showed no response. The mature embryos inoculated in media without endosperm and gave good callus quality in almost all genotypes. Similar result is reported by Hakan *et al* (2004). Day to callus induction is also varies to each other while callus size is almost similar for all genotypes. Friable and yellowish callus are produce by all genotypes but some whitish and compact callus has also produced in some tubes. The callus were sudculture and inoculate into rooting and shooting media MS+ KIN (1.2mg/L) + BAP (1.2mg/L), MS + KIN (1mg/L) + BAP (15mg/L), respectively. 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 invitro 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 + 0.5mg/L of Kinetin. Riffat *et al*. (2001) reported that, in combination of BAP or Kinetin, 2,4 –D with low concentration, shows a little callugenesis in wheat, and an increase was observed with an increase in 2,4 –D concentration, but this increase was low compared to medium containing 2,4- D alone. From the present study, it could be concluded that good and rapid shoot regeneration was on media supplement with kinetin although it took longer time

Table 1.1

Tissue culture response	Media	Mean	CD at 5%	CV %	S.E. m±	F value
Callogenesis	MS1	63.04	6.04	5.63	2.04	97.05
	MS2	76.53	11.39	8.73	3.86	22.55
	MS3	47.66	6.7	8.25	2.27	24.78
Rhizogensis	MS2	7.21	0.84	6.9	0.28	781.88
	MS3	33.43	5.11	8.98	1.73	147.82
Caulogenesis	MS2	44.74	5.18	6.79	1.75	35.27

Table 1 Analysis of variance of Responses

Mean sum of squares			
Tissue culture response	**Media**	**Genotypes**	**Error**
Establishment	MS1	304.86**	73.01
	MS2	168.46**	23.7
	MS3	203.48**	33.5
Swelling	MS1	490.53**	51.67
	MS2	164.85**	23.18
	MS3	563.71**	16.39
Callogenesis	MS1	1222.95**	12.6
	MS2	1009.06**	44.74
	MS3	383.55**	15.47
Rhizogenesis	MS2	194.37**	0.24
	MS3	1332.53**	9.01
Caulogenesis	MS2	326.52**	9.25
Number of shoot	MS2	0.69**	0
**: significance at 1% level			



A: UGANDA
B: HD2733
C: SWASN 3101

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