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Effect of Media, Growth Regulators and Genotypes on Callus Induction and Regeneration in Wheat (*Triticum aestivum*)

Hamid Rashid, [1]Rizwana Abdul Ghani, Zubeda Chaudhry,
[1]Syed Muhammad Saqlan Naqvi and Azra Quraishi
Agricultural Biotechnology Institute, National Agricultural Research Center,
Park Road, Islamabad, Pakistan
[1]Department of Biological Sciences, University of Arid Agriculture, Rawalpindi, Pakistan

Abstract: Effects of media, growth regulators and genotype were investigated for callus induction, maintenance and regeneration in wheat (*Triticum aestivum* L. cvs., Chakwal 86, Rawal 87). The percentage of callus induction in Rawal 87 was 49.58 -75.62% on Murashige and Skoog (MS) medium and 58.31-91.58% on N6 medium. For Chakwal-86, callus induction frequency ranged from 52.08- 81.04% on MS medium and 53.12- 81.02% on N6 medium supplemented with 2mg/l 2, 4 dichlorophenoxy acetic acid(2,4-D) singly, and with three concentrations (0.1, 0.5 and 1mg/L) of 6-Benzylaminopurine (BAP). BAP proved to play no role in callus induction. Sorbitol promoted callus proliferation, on N6 medium supplemented with 2mg/L 2,4-D. For plant regeneration from calli, MS medium with 0.1mg/L IAA and four concentrations (0.5, 1.0, 2.5 and 5mg/L) of BAP were tested. Regeneration frequency was greater (31.9%) in Rawal 87 on the medium containing 0.5mg/L BAP whereas 2.5mg/L of BAP were found suitable for regeneration in Chakwal 86 (15.27%).

Key words: Wheat, callus, N6 medium, MS medium, regeneration

Introduction

Various nutrient media have been used successfully for somatic embryogenesis of cereals and grains (Tomes, 1985). MS medium (Murashige and Skoog, 1962) is being tested along with different concentrations of growth regulators. It has been reported by Carman *et al.* (1987) that double concentrations of macro elements in MS medium enhanced embryogenic callus induction in comparison with other media tested. Linsmaier and Skoog (1965), (LS) medium also increased embryogenic callus induction (Mackinnon *et al.*, 1987).

N6 medium (Chu, 1978) was found suitable for anther culture by Datta and Wenzel (1987). MS medium supplemented with 2mg/L 2, 4-D was suitable for callus induction whereas addition of NAA at the rate of 1 mg/L and NaCl at the rate of 3g/L was suitable for the reculturing of callus cultures which gave good plant regeneration (Kintzios *et al.*, 1997). Addition of growth regulators in the culture medium increased plantlet regeneration frequency (Cai *et al.*, 1999; Chen *et al.*, 1999; Bahieldin *et al.*, 2000; Varshney *et al.*, 1999). Thidiazuron proved to be the best for *in vitro*

regeneration of wheat (Shan *et al.*, 2000). Different genotype gave different response to callus induction and regeneration. Several reports have been published in this regard. Elwafa and Ismail (1999) compared 16 genotypes of wheat (*Triticum aestivum)* and found Sakha 69 the most suitable on LS medium. supplemented with 2mg/L 2,4-D.

Keeping in view the importance of media recipe and response of genotype on callus induction this study was proposed to evaluate the varietal response of wheat cultivars (Rawal 87 and Chakwal 86) to callus induction and regeneration.

Materials and Methods

Materials used in this study were seeds of two arid zone wheat varieties: Chakwal 86 and Rawal 87. These were obtained from National Coordinator wheat programme at Crop Sciences Institute (CSI), National Agricultural Research Center (NARC), Islamabad. For callus induction MS and N6 media were used with 2mg/L 2,4-D singly or in combination with BAP. Each of the two media was supplemented with 0.6% agar and 3% sucrose. The medium pH was adjusted to 5.75 ± 0.05. For callus maintenance N6 medium was used in two combinations (A = N6 + 2mg/L 2,4-D) B= N6 + 2mg/L 2,4-D + 3% sorbitol.

Mature seeds were used as explant source. These were washed 2-3 times with tap water first and then with a commercially available detergent (zip) followed by several times rinsing with tap and distilled water. 100% chlorox treatment was given for 20 minutes with continuous shaking under aseptic conditions. After sterilization excessive bleach was washed off with sterilized water three times. After drying in sterilized petri plates, 2 seeds were cultured per test tube. The cultures were incubated at a temperature of 23 ± 2°C. Forty-eight replicates were made per hormonal treatment for callus induction. Experiment was conducted twice. Calli were subcultured after 4 weeks of incubation, on the maintenance medium. Embryogenic calli were further subcultured for 4 weeks. Both Embryogenic (E) and Non-Embryogenic (NE) calli were distinguished by their external appearance as described earlier by Rashid and Quraishi (1989). Calli contained both embryogenic (greenish white and compact) and non-embryogenic (Dirty white and soft) parts. Embryogenic portions were shifted to regeneration medium, after four weeks of maintenance. MS medium supplemented with 3% sorbitol, 3% sucrose, 0.6% agar, 0.1mg/L IAA and five concentrations (0, 0.1, 0.5, 2.5 and 5mg/L) BAP was used as regeneration medium.

Results and Discussion

The effect of MS and N6 media on callus induction was observed with 2,4-D single or in combination with BAP. Concentration of 2,4-D was kept constant I-e 2 mg/L and 3 concentrations (0.1, 0.5 and 1 mg/L) of BAP were used. Callus induction was observed after one week of culture in all the combinations tested, irrespective of varietal and media differences. N6 medium was found suitable for callus induction than MS. On the average the percentage of callus induction on MS medium was 61.09% whereas on N6 medium, it was 73.50%. Callus induction frequency (CIF) was greater in Rawal

87 than Chakwal 86. CIF in Rawal 87 was 58.64% on MS medium and 79.9% on N6 medium, whereas for Chakwal 86, CIF was 63.54% on MS and 67.11% on N6 medium (Table 1). The superiority of N6 medium as callus induction and maintenance medium to MS medium was also adjudged by Saeed *et al.* (1994). However their findings were for wheat anther culture and they used ½ MS medium.

BAP played no significant role in callus induction (Table 1). He *et al.* (1986) also concluded this while investigating the effect of different concentrations of cytokinin (kinetin) on callus initiation. On the other hand Ozias-Akins and Vasil (1983) reported that addition of BAP to callus initiation medium enhanced embryogenic callus formation.

On the average callus induction was greater in Rawal 87 (69.27%) than Chakwal 86 (65.32%). These results do not coincide with the results of Ullah *et al.* (2000) who earlier reported that Chakwal 86 was the most suitable while evaluating the varietal response in a similar study. Callus growth was measured by visual observation. Different grades (+++, ++, +) were made for excellent, good and average growth of calli. In Rawal 87 the percentage of excellent (+++) growth was maximum (50%) on MS medium, supplemented with 0.5mg/L BAP and 2mg/L 2,4-D, whereas the minimum or average (+) growth

Table 1: Callus induction frequency in Rawal 87 and Chakwal 86

Growth regulators		Rawal 87						Chakwal 86					
		MS			N6			MS			N6		
2, 4-D mg/l	BAP mg/l	R1%	R2%0	Mean%	R1%	R2%	Mean%	R1%	R2%	Mean%	R1%	R2%	Mean%
2.0	0.0	70	81.25	75.62	89.5	83.33	86.41	85.0	77.08	81.04	87.5	60.4	73.95
2.0	0.1	50	54.16	52.08	45.8	70.83	58.31	77.5	52.08	54.79	85.0	77.08	81.02
2.0	0.5	45	54.16	49.58	89.5	91.66	91.58	75.0	29.16	52.08	54.1	66.66	60.38
2.0	1.0	50	64.58	57.29	72.91	93.7	83.30	82.5	50.00	66.25	37.5	68.75	53.12

R1=Experiment No.1, R2=Experiment No.2

Table 2: Regeneration frequency in Rawal 87 and Chakwal 86

	Concentrations		Rawal 87					Chakwal 86				
Comb.	BAP mg/L	IAA mg/L	Total cul.	Calli with Green Center Spots	% age regenera-ble calli	No. of plantlets regene-rated	% age of regene-rated plants	Total cult-ures	Calli with Green center Spots	% age regenera-ble calli	No. of plantlets regene-rated	% age of regene-rated plants
A	0.0	0.0	72	12	16.66	0	0.00	72	12	16.66	0	0.00
B	0.0	0.1	72	0	0.00	0	0.00	72	0	0.00	0	0.00
C	0.5	0.1	72	42	58.33	23	31.90	72	36	50.00	0	0.00
D	1.0	0.1	72	30	41.66	0	0.00	72	12	16.60	7	9.72
E	2.5	0.1	72	36	50.00	0	0.00	72	54	75.00	11	15.27
F	5.0	0.1	72	60	83.33	6	8.33	72	41	56.90	0	0.00

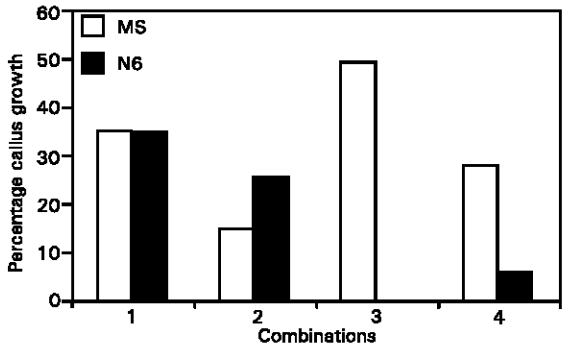

Fig. 1: Excellent (+++) callus growth from Rawal-87

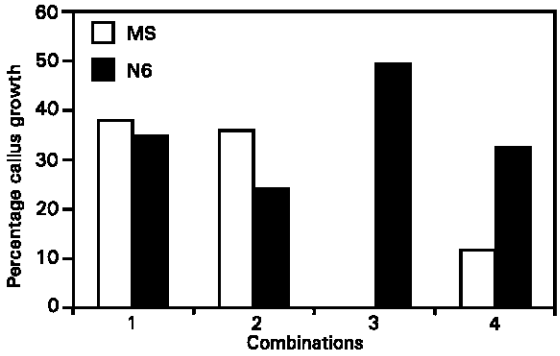

Fig. 2: Excellent (+++) callus growth from Chakwal-86.

Combinations	2,4-D (mg/l)	BAP (mg/l)
1	2.0	0.0
2	2.0	0.1
3	2.0	0.5
4	2.0	1.0

was obtained on N6 medium at the same level of 2,4-D and BAP (Fig. 1). In chakwal 86 callus growth was maximum on N6 medium supplemented with 2mg/L 2,4-D and 0.5mg/L BAP. However it was minimum at the same hormonal treatment (2mg/L 2,4-D and 0.5mg/L BAP) on MS medium (Fig. 2).

For callus maintenance effect of N6 medium with 2-mg/L 2,4-D was observed singly and in combination with 3% sorbitol. Sorbitol had a positive effect on callus proliferation, (Fig. 3). The effect of sorbitol on callus growth in wheat was investigated for the first time. However inclusion of osmoticum (sorbitol/mannitol) in plant regeneration medium contributed to greater regeneration from rice callus, as reported by Lai & Liu (1988) and Rashid ***et al.*** (2000).

Plantlet regeneration was greater in Rawal 87, MS medium supplemented with 3% sorbitol, 3% sucrose, 0.6% agar and various concentrations of growth regulators were used as regeneration medium. Calli were 11 weeks old when shifted to regeneration medium. Evidence of excessive rooting was seen after 2 weeks, whereas shoot formation occurred 4 weeks later. Excessive rooting, in both the cultivars was due to the presence of IAA (0.1 mg/L). The same findings were reported by Rashid and Quraishi (1989) while describing an efficient protocol for high frequency embryogenic callus induction & regeneration in 3 wheat cultivars. On the average, the frequency of regenerative calli (calli with green centers/spots) was maximum (83.3%) in Rawal 87 on 5 mg/L BAP whereas maximum plantlets (31.9%) regenerated on 0.5mg/L BAP. For Chakwal 86, 2.5 mg/L BAP was found suitable for the development of green spots in calluses

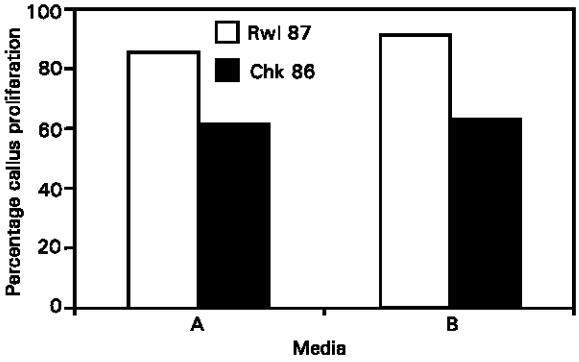

Fig. 3: Callus proliferation in Rawal-87 and Chakwal-86.A = N6 + 2,4-D (2mg/l) B = N6 + 2,4-D (2mg/l) + Sorbitol (30mg/l)

followed by plant regeneration (15.2%) (Table 2). Ullah ***et al.*** (2000) also reported this level of BAP for regeneration in Chakwal 86. On the average percentage of regenerated plants on the media tested was greater in Rawal 87 (20.11%) than Chakwal 86 (12.49%). This study has revealed a cytokinin free and auxin supplemented N6 medium as a recommended medium for callus induction. Rawal 87 which responded better to callus induction and regeneration could be used in the transformation experiments.

References

Bahieldin, A., W. E. Dyer and R. Qu, 2000. Concentration effects of dicamba on shoot regeneration in wheat. Plant Breed., 119: 437-439.

Carman, J.G., N.E. Jefferson and W. F. Campbell, 1987. Induction of embryogenic *Triticum aestivum* L. calli. I. Quantification of genotype and culture medium effects. Plant Cell Tissue Org. Cult., 10: 101 - 113.

Cai, R., K. Nakata and Y. Hirai, 1999. Plant regeneration from root callus of wheat (*Triticum aestivum* L.). Acta. Agric. Shanghai., 15: 13-17

Chen, R.M., S.M. Wen, M.L. Lang and F.S. Liang, 1999. The effect of ABA on wheat anther culture. J. Hebei. Agric. Uni., 22: 24-26.

Chu, C.C., 1978. The N6 medium and its application to anther culture of cereal crops. In: Proceedings of Symposium on Plant Tissue Culture, Science Press, Beijing, China, pp: 43-50.

Datta, S.K. and G. Wenzel, 1987 . Isolated microspore derived plant formation via embryogenesis in *Triticum aestivum* L. Pl. Sci., 48: 49-54 .

El-Wafa, A.A.A and A.E.A. Ismail, 1999. Callus induction and plant regeneration from culture of immature embryos of spring wheat. Assiut, J. Agric. Sci., 30: 13-23.

He, D.G., G. Tanner and K.J. Scott, 1986. Somatic embryogenesis and morphogenesis in callus derived from the epiblast of immature embryos of wheat (*Triticum aestivum*). Plant Sci., 45: 119-124.

Kintzios, S.E., M. Barberaki, G. Aivalakis, J. Drossopoulos and C.D. Holevas, 1997. *In vitro* morphogenetic response of mature wheat embryos to different NaCl concentration and growth regulator treatments . Plant Breed., 166: 113-118.

Linsmaier, E.M. and F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Pl., 18: 100-127.

Lai, L.K. and L.F. Liu, 1988. Increased plant regeneration frequency in water stressed rice tissue culture. Jpn. J. Crop Sci., 57: 553-557.

Mackinnon, C., G. Gunderson and M.W. Nabors, 1987. High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat (*Triticum aestivum)* and grain sorghum (*Sorghum bicolor*). *In vitro* Cellular and Developmental Biology, 23: 443-448.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Pl., 15: 473-497.

Ozias-Akins, P., and I.K. Vasil, 1983. Proliferation and plant regeneration from epiblast of *Triticum aestivum* (wheat; Gramineae) embryos. Am. J. Bot., 70: 1092-1097.

Rashid, H. and A. Quraishi, 1989. High frequency embryogenic callus induction and its regeneration in three wheat cultivars. In: A. Mujeeb, Qazi, and L.A. Sitch (eds.). Review of Advances in Plant Biotechnology, 1985-88. CIMMYT and IRRI, pp: 205-214.

Rashid, H., K. Toriyama, A. Quraishi, K. Hinata and K.A. Malik, 2000. An improved method for shoot regeneration from calli of indica rice (Basmati). Pak. J. Biol. Sci., 3: 2229-2231.

Saeed, N.A., M. A. Chowdhry and I. A. Khan, 1994. Induction of callus and organogenesis in bread wheat through anther culture. Pak. J. Agric. Res., 15:108-114.

Shan, X.Y., D.S. Li and R.D. Qu, 2000. Thidiazuron promotes *in vitro* regeneration of Wheat and Barley. *In vitro* Cellular and Developmental Biology, 36: 207-210.

Tomes, D.T. 1985. Cell culture, somatic embryogenesis: plant regeneration in maize, rice, sorghum and millet, In: S. W. J. Bright and M. G. K. Jones (Eds), Cereal Tissue and Cell Culture, Nijhoff/Dr. W. Junk Publishers, Dordrecht., pp: 175-203.

Ullah, I., H. Rashid and A. Quraishi, 2000. Varietal response of wheat, *Triticum aestivum* L. to tissue culture and assessment of somaclonal variation. Pak. J. Biol. Sci., 93: 1598-1600.

Varshney, A., S. Jain and S.L. Kothari, 1999. Plant regeneration from mature embryos of 20 cultivars of wheat (*Triticum aestivum* L. and *Triticum durum* Desf.). Cereal Res. Commun., 27: 168-170.