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Comparative Tissue Culture Response of Wheat Cultivars and Evaluation of Regenerated Plants

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Abstract: Three wheat genotypes i.e. Bakhtawar-92, Punjab-96 and Inqilab-91 were tested for their response to callus induction frequency and their subsequent regeneration on a variety of media combinations. Bakhtawar-92 appeared to be the most responsive genotype to callus induction followed by Inqilab-91 and Punjab-96. It also produced significantly higher amount of callus as compared with other genotypes. However, the medium containing 2 mg l^{-1} 2,4-D (2,4-dicholorophenoxy acetic acid) was found to be optimum for callus induction irrespective of the genotypes studied. Regeneration frequency of Bakhtawar-92 was 40% on the medium containing 0.1 mg l^{-1} IAA (Indole acetic acid) and 2.5 mg l^{-1} BAP (6-benzyl amino purine). Punjab-96 and Inqilab-91 showed regeneration of 25 and 33% on the medium supplemented with combination of 0.1 mg l^{-1} IAA and 0.5 mg l^{-1} BAP. Regenerated plants were evaluated for plant height, maturity and seed set. They had favourable significant differences from the control plants for the most important agronomic traits; plant height, days to maturity and kernels/spike etc.

Key words: Wheat, callus, regeneration, somaclonal variation, tissue culture response, callus induction

INTRODUCTION

The establishment of reliable protocols for callus production and plantlet formation is a pre-requisite for any biotechnological process. In view of the enormous efforts by various research groups, it is now possible to produce embryogenic calli of wheat easily and frequently. Formerly, it was considered recalcitrant to this technology[1].

Heritable variation is often observed in plants regenerated from tissue, cells and organ culture. Wheat has been the principal subject for investigating this phenomenon and several researchers have reported different types of somaclonal variation. Regardless the mechanism by which somaclonal variants are produced, a body of evidence have been accumulated to show that induced variation include agronomically useful traits[2]. Such variation is significant in relation to the potential role of tissue culture in genetic manipulatory and plant breeding programme.

Although tissue culture studies for wheat improvement in Pakistan has been reported Ullah *et al.*[3] but no study was initiated on the varieties grown in Northern areas of Pakistan. The present study was carried out on the varieties grown in rainfed as well as irrigated areas of Pakistan, with a view to develop protocol for efficient regeneration system and their response to tissue culture with the evaluation of somaclonal variants.

MATERIALS AND METHODS

Seeds of *Triticum aestivum* L., provided by Cereal Crop Research Institute, Pirsabak Nowshera, were used to initiate aseptic culture. Three wheat genotypes i.e. Bakhtawar-92, Punjab-96 and Inqilab-91 were tested. Sterilization and culture procedures were used as reported earlier Rashid and Quraishi[4]. Basic MS medium[6] supplemented with 2,4-D @ 1, 2, 3 and 4 mg l^{-1} was tested for callus induction frequency, where as BAP and IAA were used @ 0.5 + 0.1 mg l^{-1}, 2.5 + 0.1 mg l^{-1} 0.0 +1.0 mg l^{-1}, for plant regeneration efficiency. Twenty five replicates for each treatment per hormonal combination were used. Regenerated plants were transferred to free living conditions in glasshouse in pots having a mixture of sand, clay and farmyard manure. The plants regenerated were studied for desirable variation in agronomic traits, such as plant height, maturity and seed set.

RESULTS AND DISCUSSION

Callus induction: The Highest callus induction frequency was observed at 2 mg l^{-1} level of 2,4-D irrespective of the

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Table 1: Callus induction frequency at different levels of 2,4-D on MS media

	Bakhtawar-92		Punjab-96		Inqilab-91	
Hormonal levels of 2,4-D (mg l^{-1})	Callus frequency (%)	Callus quality	Callus frequency (%)	Callus quality	Callus frequency (%)	Callus quality
0	7.50	+	8.50	-	10.00	-
1	66.00	++	51.00	++	45.00	++
2	91.00	+++	54.00	+++	66.00	+++
3	60.00	++	52.50	++	33.33	+
4	30.00	-	24.50	+	25.00	+

- No response + Inconspicuous ++ Average +++ Good

Table 2: Embryogenic callus production on medium having 2 mg l^{-1} of 2,4-D after 2 passages

Genotype	Total	Embryogenic Calli	Embryogenic Callus (%)
Bakhtawar-92	75	46	62
Punjab-96	75	34	45
Inqilab-91	75	29	39

Table 3: Regeneration frequency of three genotypes of wheat from embryogenic calli

	Regeneration (%) BAP and IAA mg l^{-1}				
Genotype	0.00/0.00	0.5/0.1	2.5/0.1	5.0/0.1	0.00/1.0
Inqilab-91	0.00	33.00	13.00	0.00	0.00
Bakhtawar-92	0.00	10.00	40.33	0.00	0.00
Punjab-96	0.00	25.00	10.70	0.00	0.00

genotype tested. It was 91% in Bakhtawar-92, 66% in Inqilab-91 and 54% in Punjab-96. Callus induction was poor at higher levels of 2,4-D. The frequency of callus formation decreased by 33% with an increase in level of 2,4-D from 2 to 3 mg l^{-1} in Bakhtawar-92. Similar pattern was observed for Punjab-96, where as callus induction frequency was drastically decreased on the same range of hormones tested (Table 1). In this study callus induction frequency was directly related to callus growth. These findings are compatible with other workers[4,3] who reported that wheat varieties in Pakistan responded well to various levels of 2,4-D for callus formation and its maintenance. It was also found that embryogenic callus production was more in Bakhtawar-92 as compared with Punjab-96 and Inqilab-91. Embryogenic callus production in our study was directly related to callus induction frequency and callus growth.

Callus type: Two different types of calli were recognized in tested wheat varieties as embryogenic calli and non-embryogenic calli. Non-embryogenic calli remained loose, smooth and crystalline in colour in all the varieties. Eembryogenic calli was granulated, hard in texture, compact and yellowish brown with green spots and was highly differentiated in all the varieties, confirming the results of Rashid and Quraishi[4].

Embryogenic callus production: The embryogenic part of the calli were dissected and transferred to proliferation media containing 2,4-D 2 mg l^{-1}. The embryogenic callus production was assessed after two passages and it was found that Bakhtawar-92 produced maximum embryogenic callus i.e. 62 (Table 2).

The results recorded that the variety which is more responsive to callus induction also showed positive behaviour to embryogenic callus production.

Plantlets formation: The embryogenic calli of Bakhtawar-92 underwent complex organogenesis including leafy structure differentiation on transfer to the medium containing a combination of 2.5 mg l^{-1} BAP and 0.1 mg l^{-1} IAA. The calli were differentiated into leaflets with excessive rooting. Complete shoot formation was observed five weeks later. The regeneration process was similar in almost all the varieties of which maximum plantlets formation (40%) was observed in Bakhtawar-92. It was also observed that embryogenic calli developed shoots whereas non-embryogenic calli failed to regenerate. It can be inferred that a combination of 2.5 mg l^{-1} of BAP and 0.1 mg l^{-1} of IAA was an optimum level of hormones for Bakhtawar-92 and that of 0.5 mg l^{-1} of BAP and 0.1 mg l^{-1} of IAA was optimum for Punjab-96 and Inqilab-91. It means that regeneration response is variety specific.

The increasing concentrations of BAP and IAA had deleterious effects on plantlet formation (Table 3). Plantlet formation frequency was decreased with an increase in concentration levels of BAP from 0.5-5.0 mg l^{-1} in all the three varieties. The optimum doses for plantlet formation in these genotypes were IAA. 0.5 mg l^{-1} BAP and 0.1 mg l^{-1} IAA (Table 3).

Evaluation of somaclones: Evidences have accumulated over the years that the enhanced genetic variation may be obtained in plants derived from callus that have been exposed to a tissue cell culture environment without a deliberate mutagenic treatment because of the mutagenic role of some of chemicals used in medium.

In present study, all 12 somaclones differed from the parental lines in the many of the observed traits. Somaclones of almost all the varieties were characterized by small plant height as compared with control plants of these tested varieties. Similarly, somaclones of Bakhtawar-

Table 4: Agronomic and morphological traits of variants derived from Inqilab-91, Bakhtawar-92 and Punjab-96

	Inqilab-91		Bakhtawar-92		Punjab-96	
Plant traits	Control	Somaclones	Control	Somaclones	Control	Somaclones
Days to heading	60.00a	55.75b	61.00a	57.60b	63.00a	56.33b
Plant height	50.00a	45.00b	50.00a	45.30b	44.00a	41.33b
Days to maturity	90.00a	88.00a	100.00a	83.60b	91.00a	82.66b
Spike length	5.60a	5.30b	5.80a	5.86a	5.20a	5.30a
Kernels/ Spike	15.00a	18.25b	15.00a	20.80b	21.00a	21.03a

Means in a row followed by the same letter are not significantly different at 5% probability

92 showed significant earliness of five to ten days in days heading and days to maturity at 5% confidence level. However, the differences were non-significant between tissue cultured plants and those derived from seed for kernels/spikes in Bakhtawar-92. The differences in somaclones of other varieties were also significant for the traits except those for spike length in Punjab-96 and Inqilab-91 and Kernels/spike in Bakhtawar-92 as well (Table 4).

The present study showed that significant variation can be generated for a number of morphological and agronomic traits using tissue culture techniques. It is, therefore, evident that somaclonal variants can be a potential source of material for generating improved genotypes. These findings are in agreement with those of Maddock *et al.*[5] and Mohamand and Nabors[2].

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