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Varietal Response of Wheat, *Triticum aestivum* L. To Tissue Culture and Assessment of Somaclonal Variation

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Abstract: Three wheat genotypes i.e chakwal-86, NR-58 and Inqilab-91 were tested. Chakwal-86 appeared to be the most responsive genotype to callus induction followed by NR-58 and Inqilab-91. It also produced significantly higher amount of callus compared to other genotypes. Callus induction frequencies varied from 66.65 to 100 percent in Chakwal 86, 49 to 75.80% in Inqilab-91 and 60.90 to 75.50% NR-58 on various levels of 2,4-D (2,4- dichlorophenoxy Acetic Acid). However, medium containing 2 mg of 2,4-D was found to be optimum for callus induction. Regeneration frequency of Chakwal-86 was 33.33% on the medium containing 0.1 mg of IAA (Indole Acetic Acid) and 2.5 mg of BAP (6-Benzyle Amino Purina). While NR-58 and Inqilab-91 showed regeneration percentage of 40 and 25% respectively on medium having 0.1 mg of IAA and 0.5 mg of BAP. Regenerated plants were transferred to free living conditions. Regenerated plants were evaluated for plant height, maturity and seed set. Regenerated plants showed favourable significant differences from control plants at 5 96 confidence level for most of the studied traits; such as plant height, days to maturity and etc.

Key words: Wheat callus regeneration somaclonal variation

Introduction

Various conventional methods are being employed for the improvement of wheat to maximize wheat yield by development of improved lines/varieties and to mitigate the effect of various factors which perturb wheat productivity. These factors are diseases, pests, climatic influences and changing consumer demands. The combination of these factors or a single may reduce the life of a new variety to 4-5 years. Wheat breeding is therefore a continuous endeavour. The success of wheat breeding lies in the extent of genetic variability available for a trait to be improved and its ingenious exploitation (Mentewab *et al*., 1997).

Heritable variation is observed in plants regenerated from tissue, cells or organ culture (Larkin and Scowcroft, 1981). Wheat has been the principal subject for investigating this phenomenon and several researchers have reported different types of somaclonal variations (Larkin and Scowcroft, 1981; Ahloowalia and Sherington, 1985; Maddock *et al*., 1983; Chen, 1985). Ryan *et al*. (1987) have reported significant variation in characters such as plant height, grain number per spike, kernel weight, yield, total dry weight and harvest index. The origin of this source of variation is however, not yet known. It may be due to exposure of tissues to various environments (Lazar *et al*., 1983; Henry and De Buyser, 1985). Regardless of the mechanism by which somaclonal variation is produced, evidences have accumulated that such variation does include agronomically useful type of variants (Mohmand and Nabors, 1990).

The application of many biotechnological methods in cereal improvement involves the establishment of standardized protocol for the production of embryogenic callus and plant regeneration. The regeneration of superior plants depends upon the production of embryogenic callus which is then used as basis for all techniques of biotechnology. Most commonly used source of embryogenic callus culture of wheat is seed as reported by Scott *et al*. (1990) but other explant sources can also be used (Mohmand and Nabros, 1990). In comparison with rice *(Oryzae sativa* L.), wheat remains a species needing additional research regarding somaclonal variants production and genetic transformation. In the present study the main aim was to establish a protocol for callus induction and plant regeneration for three wheat genotypes viz. Chakwal-86, Inqilab-91 and NR-58. The second objective was to screen wheat varieties for tissue culture potential and gather evidence of somaclonal variation from regenerated plants.

Materials and Methods

Seeds of *Triticm aestivum* L., provided by the National Coordinated programme on wheat, National Research Centre (NARC) Islamabad, were used to initiate aseptic cultures. Three wheat genotypes (Chakwal-86, NR-58 and Inqilab91) were tested. Sterilization and culture procedures were used as described before by Rashid and Quraishi (1989). Basic MS media supplemented with 2,4-D at the rate of 1 mg/l, 2mg/l, 3mg/l and 4mg/l was tested for callus induction frequescy, whers as BAP and IAA at the rate of 05 mg + 0.1 mg, 2.5 mg + 0.1, 5.0 mg + 0.1 mg, 0.0 mg +1.0 mg was for plant regeneration efficiency, Seventy two replicates for each treatment per hormonal combination were used. Regenerated plants were transferred to free living conditions in glasshouse in pots having mixture of sand, clay and farm yard 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, morphology and growth pattern of calli varied with the donor plants used and quantity of 2, 4-D in culture medium as reported earlier (Marciniak *et al*., 1997). Medium containing 2 mg/l of 2,4-D was found to be optimum for callus formation in our study. Callus induction frequency with good callus growth was achieved on MS medium containig 2,4-D 2 mg/l irrespective of the varieties tested. Low callus growth was obtained in all varieties tested at high concentrations (3 and 4 mg/l) of 2,4-D (Table 1).

This is in line with findings already reported (Sunderland and Dunewell, 1973). Callli obtained on this medium were mostly

Table 1: Callus induction frequency of three genotypes of wheat

Hormonal levels of 2.4-D	Callus form frequency	Growth Quality	Callus form Frequency	Growth Quality	Callus form frequency	Growth Quality
1 mg/l	97.0	+ +	70.5	+ +	75.8	+
2 mg/l	80.2	+ + +	66.2	+ + +	65.0	+ + +
3 mg/l	86.7	+	60.9	+	68.3	+
4 mg/l	85.8	+	66.0	+	49.1	+

+ Inconspicous = + + Average = + + + Good

Table 2: Regeneration frequency of three genotypes of wheat from embryogenic calli

Genotypes	BAP mg/l and IAA mg/l				
	0.00/0.00	0.5/0.1	2.5/0.1	5.0/0.1	0.00/1.0
Chakwal-86	0.00	10.00	33.33	0.00	0.00
NR-58	0.00	40.00	0.00	0.00	0.00
Inqilab-91	0.00	25.00	10.70	0.00	0.00

Table 3: Comparision between control and regenerated plants of chakwal-86, NR-58 and Inqilab-91

Genotypes	Plants	Days to 50% heading	Plant height (cm)	Days to maturity	Spike length (cm)	Kemeis/Spika
Chakwal-86						
	Cont.	61	50.00	100	5.6	15
	S. Variants 1	57	45.75	80	5.9	20
	S. Variants 2	58	43.25	82	5.8	18
	S. Variants 3	55	44.50	83	5.7	17
	S. Variants 4	60	46.00	80	5.7	21
	S. Variants 5	58	44.00	85	5.9	23
NR-58	Cont.	63	44.00	91	6.0	21
	S. Variants 1	56	42.00	83	5.8	25
	S. Variants 2	55	45.00	80	5.7	19
	S. Variants 3	58	48.00	85	5.9	22
	S. Variants 4	59	43.00	87	5.9	23
Inqilab-91	Cont.	59	48.00	90	5.2	18
	S. Variants 1	53	45.00	88	5.5	17
	S. Variants 2	55	46.00	88	5.6	20
	S. Variants 3	68	44.00	83	5.3	23

Table 4: Agronomic and morphological traits of variants derived from C-86, NR-58 and 1-91

Plant Traits	C-86		NR-58		Inqilab-91	
	Control	Somaclones	Control	Somaclones	Control	Somaclones
Days to 56 heading	61.2a	57.6b	62.4a	57.4b	58.8a	54.6b
Plant height	50.8a	44.7b	44.8a	42.8b	46.9a	44.00b
Detya to maturity	99.4a	82.0b	91.4a	84.0b	89.88	85.4b
Spike length	5.70a	5.8h	6.06a	5.86b	5.08a	5.66b
Karnal/Splke	15a	19.8b	21a	22.25	18a	22.66b

Means in a now followed by same letter do not differ significantly at 5% then probability

compact embryogenic in nature. Callus frequencies of 80.2, 00.3 and 66% were obtained respectively for Chakwal-86, Inaliab-91 and NR-58 on medium having 2 mg/l (Table 1). It ie also clear that concentration levels of 2, 4-D also affected callus induction in wheat (Colins *et al*., 1978). The ambryogenic calli of all the genotypes were cultured on the MS medium containing various levels of BAP and IAA. Regeneration frequency of C-86 was 33.33% on medium containing 2.5 mg/l of BAP and 0.1 mg/l of IAA while regeneration frequescies of 1-91 and NR-58 were 25 and 40% reepectivily. Ingilab-91 and NR-58 responded well to medium with 0.5 mg/l of BAP and 0.1 mg/l of IAA. The embryogenic calli of C-86 underwent comples organogeneele including leaf structre differenciatation on transfer to media containing BAP at 2-5 mg/l with IAA 0.1 mg/l The calli were differentiated into leaflet initiation with excessiverooting. Complete shoot formation was observed five weeks later. Mode of regeneation was almost similar in all the genotypes, although maximum plantlet formation (26.64%) was obtained in Chakwal-86 as compared to two other varieties (16.4%-20%). It was found that E. Ccelli produced plant while NE-calli failed to regenerate (Table 2). Same findings were reported earlier by Rashid and Quraishi (1989).

Five somaclones of Chskwal-86, four of NR-58 and three of Inqilab were selected. All of them have less plant height as compared to their control varieties. Similarly, somaclones of Chakwal-86 showed 15 to 20 days early maturity as compared the control variety, whereas the somaclones of Ingilab-91 has no significant differences in days to maturity from the control variety. The somaclones of NR-58 have less significant differences to their control (Table 3 and 4). Table 4 shows the superiority of regenerated plants over the control ones. It is therefore, evident that somaclonal variants can be a potential sources of material for the evolution of new and improved genotypes. This is in line with a number of reports published so far (Mohmand and Nabors, 1990). Unfortunately the frequency of regeneration was low.

Variation showed by somaclonese may be of great practical

help to breeders. The phenomenon of plant regeneration shout be studied further inorder to determine the genetic base of these variations for utilization in different breeding programmes.

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