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HIGH FREQUENCY REGENERATION SYSTEM OPTIMIZATION FOR WHEAT CULTIVAR INQILAB-91

ASMA AFZAL[1], HAMID RASHID[2*], M. HAROON KHAN[2], ZUBEDA CHAUDHRY[3] AND SALMAN A. MALIK[1]

[1]*Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Department of Bioinformatics, Mohammad Ali Jinnah University, Islamabad*
[3]*Department of Botany, Haraza University, Mansehra.*

Abstract

In the present study, a high efficiency regeneration system was developed in wheat (*Triticum aestivum* L.) cv. Inqilab-91. Mature embryos were taken as explant source and inoculated on MS media for callus induction. MS media with different concentrations of 2, 4-D (2mg/l, 3mg/l and 4mg/l) were used for callus induction. Maximum percentage (76.04%) of callus induction was achieved with 3mg/l of 2, 4-D whereas 53.12% and 56.25% of calli were formed with 2mg/l and 4mg/l of 2, 4-D respectively. Four different hormones (IAA, BAP, Kn and 2ip) were used in different concentrations and combinations in MS medium for regeneration from calli of mature embryos. The regeneration frequency (46.66%) was obtained with 0.1mg/l of IAA and 0.5mg/l of BAP. Different concentrations of Kn (0mg/l, 0.5mg/l, 1.0mg/l) were added to the optimized concentration of IAA (0.1mg/l) and BAP (0.5mg/l) to achieve more regeneration frequency. Regeneration frequency (56.66%) was obtained with 0.5mg/l of Kn along with IAA (0.1mg/l) and BAP (0.5mg/l). 60% regeneration was achieved on medium containing IAA 0.1mg/l and Kn 0.5mg/l. Different concentrations of 2ip (0mg/l, 0.5mg/l, 1.0mg/l and 1.5mg/l) were also tested along with IAA and Kn and regeneration frequency of 65% with IAA (0.1mg/l), Kn (0.5mg/l) and 2ip (1.0mg/l) which was maximum in all the combinations experimented before. High frequency system ranged from 46.66 to 65% depending upon the type of hormone concentration used was optimized. We were successful to achieve high frequency regeneration by manipulating hormonal type and concentration which were not reported earlier.

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 (Rahman *et al.*, 2008). Globally, it is the second largest cereal crop behind maize. A number of environmental factors such as temperature, moisture, soil and light intensity affect the growth and yield of wheat (Shah *et al*., 2003).

In recent years biotechnology is emerging as one of the latest tools in agricultural research and is contributing towards the development of novel methods to genetically alter and control plant development, performance and its products (Patnaik & Khurana, 2001). Transformation of cereal crops is a powerful research tool for gene discovery and function to investigate genetically controlled traits and is becoming a key element in the process of varietal improvement (Jones *et al*., 2005). Wheat was among the last of the major crops to be transformed (Vasil *et al*., 1992). Furthermore, transformation still remains more difficult for wheat being more genotype dependent and having lower efficiency (Shewry & Jones, 2005).

Plant regeneration frequency depends upon specific combination of media used and genotype of wheat. Similarly, age of callus also play critical role in this regard (Saad *et al*., 2004). Inqilab-91 is cultivated in 70% of irrigated area of Punjab and is high yielding lodging resistant and general purpose variety suitable for rich soils under normal and late planting conditions (Mujahid, 2004). The objective of the present study was to optimize a high frequency regeneration protocol for wheat cultivar Inqilab-91 which will further lead for developing transformation protocols in this cultivar.

*Corresponding author: E-mail: drhamid@jinnah.edu.pk; Ph: 051-111878787

Materials and Methods

Mature embryos of wheat, *Triticum aestivum* L. cv. Inqilab-91, were used throughout this study. MS (Murashige & Skoog, 1962) media supplemented with different concentrations of 2, 4-D (2mg/l, 3mg/l and 4mg/l) was used for initiating callus from mature embryos. The mature seeds were taken and washed with running tap water for 15-20 minutes and then rinsed with distilled water. The seeds were sterilized with 50% chlorox, along with 1-2 drops of Tween-20, for 20 minutes with regular shaking. The seeds were washed with autoclaved distilled water three times at regular interval of 5 minutes. The embryos were removed with the help of scalpel and inoculated in MS medium. The cultures were incubated in growth room at 25±1°C for 20 days for proliferation. After 20 days, the calli derived from mature embryos were shifted to maintenance medium i.e., MS medium containing 3mg/l 2, 4-D. After 20 days, the calli were shifted to regeneration medium i.e., MS media + 3% sucrose, 3% sorbitol + 0.2% casine hydrolysate and 0.4% gelrite along with different combinations and concentrations of four different (IAA, BAP, Kn and 2ip) growth hormones. These cultures were placed in growth room at 25°C with 16-18 hrs of photoperiod and light intensity of 16 m.mol.m.s.

Results and Discussion

Callus induction: For callus induction, MS media along with different concentrations of 2, 4-D (2mg/l, 3mg/l and 4mg/l) were used. Maximum percentage (76.04%) of callus induction was achieved with 3mg/l of 2, 4-D in MS media whereas 53.12% and 56.25% of calli were formed with 2mg/l and 4mg/l of 2,4-D respectively (Table 1, Fig. 1). Turhan & Baser (2004) obtained similar results with 4mg/l of 2, 4-D + 1mg/l NAA. Shah *et al*., (2003) observed callus induction at 3.5mg/l and good callus induction at 3mg/l of 2, 4-D. These results match with the present study in which 3mg/l was found good for callus induction. Minor differences are due to different genotypes used. Sarker & Biswas (2002) tested different concentrations of 2, 4-D for callus induction and found 6mg/l of 2, 4-D as best concentration for callus induction from seeds and mature embryos which are quite in contrast with present study.

Regeneration: For regeneration of plants from calli of mature embryos, four different hormones viz., IAA, BAP, Kn and 2ip were used in different concentrations and different combinations in MS medium with 3% sucrose, 3% sorbitol, 0.2% casine hydrolysate and 0.4% gelrite as gelling agent.

Different concentrations and combinations of IAA (0mg/l, 0.1mg/l, 0.2mg/l & 0.3mg/l) and BAP (0mg/l, 0.5mg/l, 1.0mg/l, 1.5mg/l & 2mg/l) were used in one experiment to optimize the regeneration combination. The maximum regeneration was obtained with 0.1mg/l of IAA and 0.5mg/l of BAP. The percentage of regeneration was 46.66% (Table 2). Rashid *et al*., (2002) also reported 0.1mg/l of IAA and 0.5mg/l of BAP as best combination for maximum plantlet regeneration (31.9%) in *Triticum aestivum* L., cv Rawal-87. However, the results of present study coincide with the results of Alizadeh *et al*., (2004) who reported 1mg/l of BAP, 0.2mg/l IAA and 0.2mg/l of 2, 4-D a best combination for shoot regeneration in embryo explants and 0.2mg/l 2, 4-D and 2mg/l BAP a best combination for shoot regeneration in excised embryo explants.

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

Conc. of 2, 4-D (mg/l)	Total no. of explants	Total no. of uncontaminated explants	Number of calli	Percentage of calli (%)	Number of embryogenic calli	Percentage of embryogenic calli (%)
2	192	164	102 ± 8.83	53.12	72 ± 8.34	37.5
3	192	154	146 ± 5.38[a**]	76.04	130 ± 5.68[c**]	67.70
4	192	150	108 ± 8.04[b***]	56.25	24 ± 5.68[d***e**]	12.5

a: Number of calli induced at 2mg/l vs 3mg/l of 2, 4-D; b: Number of calli induced at 3mg/l vs 4mg/l of 2, 4-D
c: Number of embryogenic calli induced at 2mg/l vs 3mg/l of 2, 4-D; d: Number of embryogenic calli induced at 2mg/l vs 4mg/l of 2, 4-D
e: Number of embryogenic calli induced at 3mg/l vs 4mg/l of 2, 4-D; ** $p<0.01$, *** $p<0.001$

Table 2. Effect of different concentration of IAA and BAP on regeneration of wheat.

Conc. of IAA (mg/l)	Conc. of BAP (mg/l)	No. of calli	Growth & proliferation	Differentiation	Green spot	Plantlet formation	Percentage of plantlet formation (%)
0	0	30	17	11	0	0.250 ± 0.500	0
0.1	0	30	6	6	4	0.250 ± 0.500	0
0.2	0	30	0	0	0	0.250 ± 0.500	0
0.3	0	30	15	9	9	0.250 ± 0.500	0
0	0.5	30	12	12	12	0.250 ± 0.500	0
0.1	0.5	30	22	19	17	14 ± 3.40[a**]	46.66
0.2	0.5	30	15	7	0	0.250 ± 0.500	0
0.3	0.5	30	8	8	0	0.250 ± 0.500	0
0	1	30	8	0	0	0.250 ± 0.500	0
0.1	1	30	9	5	3	0.250 ± 0.500	0
0.2	1	30	4	0	0	0.250 ± 0.500	0
0.3	1	30	4	0	0	0.250 ± 0.500	0
0	1.5	30	4	4	4	0.250 ± 0.500	0
0.1	1.5	30	0	0	1	0.250 ± 0.500	0
0.2	1.5	30	0	0	0	0.250 ± 0.500	0
0.3	1.5	30	8	8	4	0.250 ± 0.500	0
0	2	30	3	0	0	0.250 ± 0.500	0
0.1	2	30	3	3	3	0.250 ± 0.500	0
0.2	2	30	4	4	3	0.250 ± 0.500	0
0.3	2	30	0	0	0	0.250 ± 0.500	0

a: Plantlet formation at all other concentrations of IAA & BAP vs 0.1mg/l of IAA and 0.5mg/l of BAP
** $p<0.01$

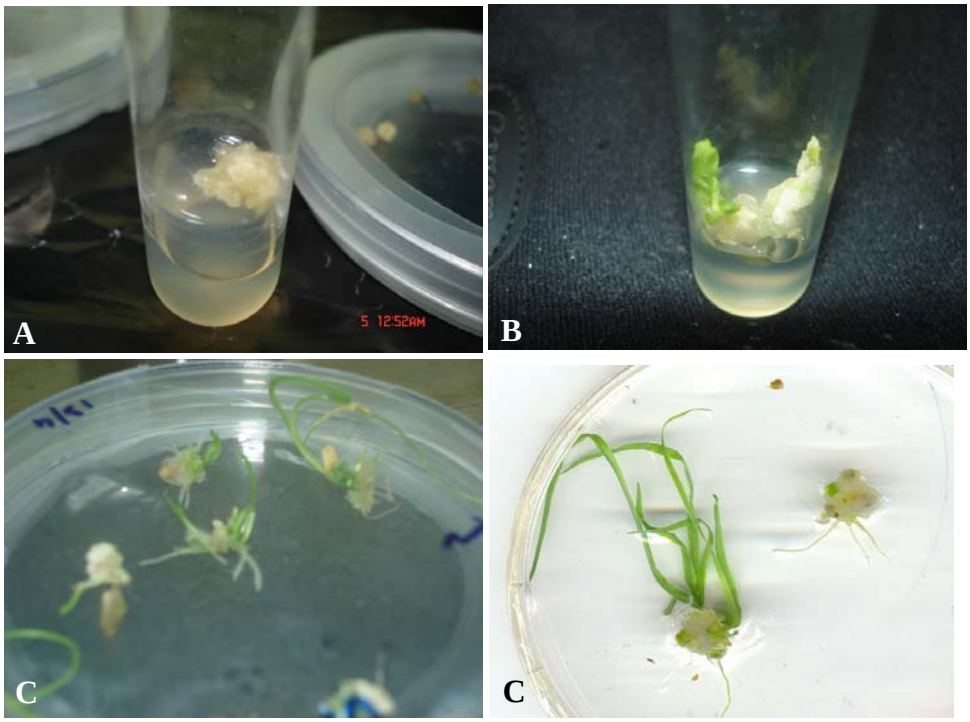

Fig 1. Regeneration of Inqilab-91 on MS medium with different combinations of growth regulators.
A: Callus induction at 3mg/l of 2, 4-D
B: Regeneration of mature embryo derived calli of wheat at 0.1mg/l IAA and 0.5mg/l BAP
C: Regeneration of mature embryo derived calli at 0.1mg/l IAA, 0.5mg/l BAP and 0.5mg/l Kn
D: Regeneration of mature embryo derived calli of wheat at 0.1mg/l IAA, 0.5mg/l Kn and 1.0mg/l 2ip

The optimized concentration of IAA (0.1mg/l) and BAP (0.5mg/l) were further tested with different concentrations of Kn (0mg/l, 0.5mg/l, 1.0mg/l). The best regeneration frequency (56.66%) was obtained with 0.5mg/l of Kn along with IAA (0.1mg/l) and BAP (0.5mg/l) (Tables 3 & 4). These results are in accordance with the results of Sarker & Biswas (2002) who also used 0.5mg/l of Kn and obtained best results.

Different concentrations of 2ip (0mg/l, 0.5mg/l, 1.0mg/l and 1.5mg/l) were tested along with optimized concentrations of IAA (0.1mg/l) and Kn (0.5mg/l) while BAP was eliminated. The maximum regeneration frequency (65%) was obtained with IAA (0.1mg/l), Kn (0.5mg/l) and 2ip (1.0mg/l) [(Table 5)] which was maximum in all the combinations experimented before. The effect of 2ip was better, as 2ip promoted root formation. Many shoots were found originating from single callus when 2ip was used in regeneration medium. So the addition of 2ip produced better effect.

Table 3. Effect of different concentrations of Kinetin along with IAA (0.1mg/l) and BAP (0.5 mg/l) on regeneration of wheat.

Conc. of kinetin (mg/l)	No. of calli	Growth & proliferation	Differentiation	Green spot formation	Plantlet formation	Percentage plantlet formation (%)
0	30	22	19	17	14 ± 3.4	46.66
0.5	30	26	26	22	17 ± 3.59	56.66
1.0	30	14	10	10	6 ± 1.49[a**b**]	20

a: Plantlet formation at 0mg/l Kn vs 1.0mg/l Kn
b: plantlet formation at 0.5mg/l Kn vs 1.0mg/l Kn
**p<0.01

Table 4. Effect of different concentrations of kinetin, IAA and BAP on regeneration of wheat.

Conc. of IAA (mg/l)	Conc. of BAP (mg/l)	Conc. of kn (mg/l)	No. of calli	Growth & proliferation	Differentiation	Green spot	Plantlet formation	Percentage of plantlet formation (%)
0	0.5	0.5	30	24	18	14	11±2.14	36.66
0.1	0	0.5	30	27	24	24	18±2.46[a**]	60
0.1	0.5	0	30	22	19	17	14±3.4	46.66

a: Plantlet formation at 0mg/l IAA, 0.5mg/l BAP, 0.5mg/l Kn vs 0.1mg/l IAA, 0mg/l BAP, 0.5mg/l Kn
*p<0.05, **p<0.01

Table 5. Effect of different concentrations of 2ip along with IAA (0.1mg/l) & Kinetin (0.5mg/l) on regeneration of wheat.

Concentration of 2ip (mg/l)	Number of calli	Growth & proliferation	Differentiation	Green spot	Plantlet formation	Percentage of plantlet formation (%)
0	20	15	15	14	12 ± 2.16	60
0.5	20	7	4	3	0.250 ± 0.500[a**]	0
1.0	20	16	15	15	13 ± 3.16[c**]	65
1.5	20	12	12	11	7 ± 2.08[b*d*e*]	35

a: Plantlet formation at 0mg/l 2ip vs 0.5mg/l 2ip; b: Plantlet formation at 0mg/l 2ip vs 1.5mg/l 2ip
c: Plantlet formation at 0.5mg/l 2ip vs 1.0mg/l 2ip; d: Plantlet formation at 0.5mg/l 2ip vs 1.5mg/l 2ip

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