African Journal of Biotechnology Vol. 8 (20), pp. 5565-5569, 19 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Optimization of callus induction and regeneration system for Pakistani wheat cultivars Kohsar and Khyber-87

Sabahat Noor[1], Ghulam Muhammad Ali[2]*, Umer Rashid[2], Muhammad Arshad[1], Shoukat Ali[2] and Yusuf Zafar[2]

[1]Department of Botany, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan.
[2]Plant Biotechnology, NIGAB, National Agriculture Research Center Park Road Islamabad, Pakistan.

Accepted 30 June, 2009

Wheat is a member of family Poaceae. It is the major staple food of Pakistan. The present study was done to improve the regeneration of two commercially grown wheat varieties Kohsar and Khyber-87. Mature embryos were used as explants. Five different concentrations of 2,4-D; 2, 2.5, 3, 3.5 and 4 mg/L were used for callus induction. For regeneration, initially different concentrations (0.1 to 0.2) of IAA (indole-3-acetic acid) and BAP (6-benzylaminopurine) were experimented. The best combination of these hormones that is, 0.1 mg/L IAA and 0.5 mg/L BAP were further subjected to experimentation along with different concentrations of kinetin; 0.1, 0.2, 0.3, 0.4, 0.5 and 1 mg/L. Maximum calli of Kohsar (83.3%) was obtained at 3 mg/L 2,4-D whereas for Khyber-87 maximum callus induction (71.70%) was obtained at 3.5 mg/l 2,4-D. The maximum regeneration of both Kohsar and Khyber-87 (80.5 and 62.2%, respectively) were obtained at the combinations of 0.1 mg/L IAA, 0.5 mg/L BAP and 0.5 mg/L kinetin.

Key words: Kohsar, Khyber-87 and wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a main cereal crop and is the staple food of many countries including Pakistan. It is one of the most important domesticated crops grown around the world. Among the food crops, wheat is one of the most abundant sources of energy and protein for the world population (Harlan, 1981). However wheat is subjected to various biotic (fungal, bacterial and viral diseases) and abiotic (drought, salt) stresses that cause major yield losses. In Pakistan the most destructive diseases of wheat are leaf rust, stem rust, loose smut and leaf blight that cause losses up to 50% of yield.

The development of resistant cultivars appears to be the most effective and economical method for controlling the diseases. Therefore the genetic improvement of wheat has received considerable attention over the years from plant breeders with the purpose of increasing grain yield and to minimize crop loss due to attack of various pests and pathogens. For this purpose genetic transformation techniques are used to improve different crop plants. Scientists have successfully obtained transgenic plants of the same family (Poaceae) including rice (Mohanty et al., 1999) and maize (Wang et al., 2000) by using transformation techniques. Mooney and Goodwin (1991) and Peters et al. (1999) also reported *Agrobacterium* mediated wheat transformation.

For successful gene transformation system regeneration from highly embryogenic somatic cultures or cell suspension must be developed. Thus, the success of plant transformation largely depends on effective regeneration protocols. Therefore before transformation experiments, the regeneration protocol is needed to develop for selected wheat varieties. In the present study *in vivo* plant regeneration protocol was established that could be further used for *Agrobacterium* mediated wheat transformation.

*Corresponding author. E-mail: drgmali@yahoo.ca.

Abbreviations: 2,4-D, 2,4-Dichlorophenoxy acetic acid; **BAP**, 6-benzylaminopuri; **IAA**, indole-3-acetic acid; **MS**, Murashige and Skoog (1962) medium.

MATERIALS AND METHODS

Plant

Seeds of two local wheat (*T. aestivum* L.) varieties, namely Kohsar

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

Variety	2,4-D concentration (mg/L)	Number of seeds inoculated	Number of calli produced	% of calli
Kohsar	2	180	64	35.5
	2.5	180	78	43.3
	3	180	150	83.3
	3.5	180	84	46.6
	4	180	60	33.3
Khyber-87	2	180	37	20.5
	2.5	180	62	34.4
	3	180	88	48.8
	3.5	180	140	77.7
	4	180	74	41.1

and Khyber-87, were collected from National Agriculture Research Center (NARC) Islamabad.

Surface sterilization and callus induction

Seeds were washed with 2-3 drops of chemical detergent (Tween-20) for 3 min and then thoroughly rinsed with running tap water for 3-4 times. They were surface sterilized with 70% ethanol for 30 s. Finally surface disinfections were done with 40% Clorox (Hypocaloric acid) for 20 min followed by several washes with autoclaved distilled water. Then they were dried and cultured in tubes.

MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose as a carbon source and solidified with 0.6% (w/v) agar was used. The pH of MS medium was adjusted at 5.75 before autoclaving at 121°C at 105 kPa for 20 min. The seeds of two wheat varieties were used for callus induction. Seeds were cultured in test tubes containing MS medium supplemented with different concentrations of 2,4-D (2, 2.5, 3, 3.5 and 4 mg/L). These cultures tubes were incubated under 16/8 h light/dark cycles at 25°C in a growth chamber. After three weeks calli were excised and shifted to regeneration medium.

Regeneration

The three weeks old calli were dissected into small pieces and were transferred to regeneration medium. Regeneration media was supplemented with various combinations and concentrations of IAA, BAP and kinetin. Regeneration was divided into two steps. In first step the effect of different concentrations of IAA and BAP were tested on regeneration frequency, whereas in the second step, the best-known combination of these hormones was experimented along-with different concentrations of kinetin (0.1, 0.2, 0.3, 0.4 and 0.5 mg/L).

RESULTS AND DISCUSSION

Callus induction

For callus induction different concentrations of 2,4-D (2, 2.5, 3, 3.5 and 4 mg/L) were used. Among these concentrations 3 mg/L 2,4-D showed the best response for callus induction (83.3%) in Kohsar. As the concentration of 2,4-D was increased from 3 to 4 mg/L, the percentage of calli was drastically reduced from 83.3 to 33.3% (Table 1). On the other hand Khyber-87 showed a different response as 3.5 mg/L 2,4-D was found to be the best for callus induction in Khyber-87. Percentage of callus induction was increased from 20.5 to 77.7% with an increase in 2,4-D concentration from 2 to 3.5 mg/L. Any further increase in 2,4-D concentration would decreased the percentage of calli. At 4 mg/L 2,4-D the percentage of calli was 41.1%. At 3 and 3.5 mg/L 2,4-D, Kohsar and Khyber-87 respectively produced compact, nodular, whitish to creamy in color and larger calli than those obtained at other concentrations (Figures 1 and 2). These results do not coincide with the results of Turhan and Baser (2004) who obtained best callus induction with 4 mg/L 2,4-D and 1 mg/L NAA. Sarker and Biawas (2002) also found good callus induction at 6 mg/L 2,4-D in wheat seeds. However Shah et al. (2003) observed excellent callus induction at 3.5 mg/L and good callus induction at 3 mg/L 2,4-D. These results match with the present study in which 3.5 mg/L 2,4-D was found good for callus induc-

Figure 1. Callus induction of Kohsar with3 mg/L 2,4-D.

Table 2. Effect of different concentrations of IAA and BAP on regeneration.

Variety	IAA (mg/L)	BAP (mg/L)	Number of calli inoculated	Number of calli showing green spots	% of regeneration
Kohsar	0	0.5	92	0	0
	0.1	0.5	92	66	71.7
	0.2	0.5	92	50	54.3
	0	1.5	92	0	0
	0.1	1.5	92	8	8.6
	0.2	1.5	92	2	2.2
	0	2.5	92	0	0
	0.1	2.5	92	0	0
	0.2	2.5	92	0	0
Khyber-87	0	0.5	92	0	0
	0.1	0.5	92	40	43.4
	0.2	0.5	92	30	32.6
	0	1.5	92	0	0
	0.1	1.5	92	13	14.1
	0.2	1.5	92	4	4.3
	0	2.5	92	0	0
	0.1	2.5	92	0	0
	0.2	2.5	92	0	0

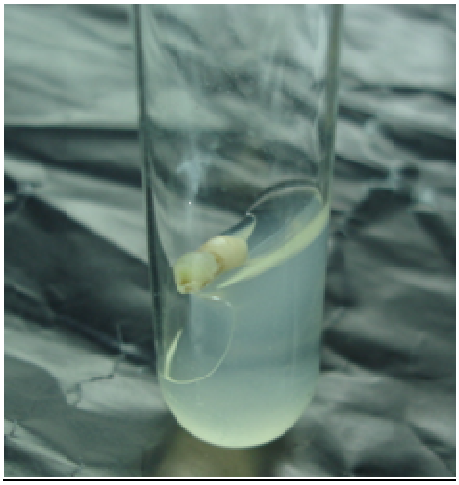

Figure 2. Callus induction of Khyber-87 with 3.5 mg/L 2,4-D.

Figure 3. Regeneration of Kohsar with 0.1 mg/L IAA and 0.5 mg/L BAP.

tion in Khyber-87 and 3 mg/L for Kohsar. These variations may be due to different genotypes of wheat varieties as each variety show a different response to tissue culture techniques.

Effect of IAA and BAP on regeneration

Twelve different combinations of IAA and BAP were used for regeneration from mature embryo derived calli. Among these combinations, 0.1 mg/L IAA and 0.5 mg/L BAP showed the best response for regeneration in both Kohsar and Khyber-87 (Figures 3 and 4). In this combination, about 71.7 and 43.4% calli showed green spots in Kohsar and Khyber-87, respectively (Table 2). However there was no regeneration without IAA. Similarly at high concentration of BAP, the percentage of regeneration was reduced drastically. It shows that high doses of BAP might have inhibitory effects on regeneration. Rashid et al. (2002) also reported the same 0.1 mg/L IAA and 0.5 mg/L BAP as best combination for maximum plantlet formation (31.95%) in wheat *cv*. Rawal-87. However the results of present study do not coincide with the results of Alizadeh et al. (2004) who found best shoot regeneration in wheat at 0.2 mg/L IAA, 1 mg/L BAP and 0.2 mg/L 2,4-D. Similarly Sarker and Biawas (2002) reported 0.5 mg/L

Table 3. Effect of different concentrations of kinetin on regeneration along-with 0.1 mg/L IAA and 0.5 mg/L BAP.

Variety	Concentration of kinetin (mg/L)	Number of calli inoculated	Number of calli showing green spots	% of regeneration
Kohsar	0.1	82	5	6.0
	0.2	82	13	15.8
	0.3	82	27	32.9
	0.4	82	58	70.7
	0.5	82	66	80.5
Khyber-87	0.1	82	12	14.6
	0.2	82	15	18.2
	0.3	82	28	34.1
	0.4	82	35	42.6
	0.5	82	51	62.2

Figure 4. Regeneration of Khyber-87 with 0.1 mg/L IAA and 0.5 mg/L BAP.

Figure 5. Regeneration of Kohsar with 0.1 mg/L IAA, 0.5 mg/L BAP and 0.5 mg/L kinetin.

IAA, 0.5 mg/L BAP and 40 mg/L tyrosine as best combination for shoot regeneration in wheat.

Effect of kinetin on regeneration

The best combination of IAA (0.1 mg/L) and BAP (0.5 mg/L) was further experimented with 6 different combinations of kinetin for best regeneration. Addition of kinetin was found to have a positive effect on regeneration. It was observed that 0.5 mg/L kinetin along with 0.1 mg/L IAA and 0.5 mg/L BAP produced the highest regeneration frequency in both varieties (Table 3). When the concentration of kinetin was decreased from 0.5 to 0.1 mg/L the percentage of regeneration was also decreased from 80.60 and 62.2% to 6.0 and 14.6% in Kohsar and Khyber-87, respectively.

Figure 6. Regeneration of Khyber-87 with 0.1 mg/L IAA, 0.5 mg/L BAP and 0.5 mg/L kinetin.

Conclusion

The best combination for highly efficient regeneration of Kohsar and Khyber-87 is 0.1 mg/L IAA, 0.5 mg/L BAP and 0.5 mg/L kinetin (Figures 5 and 6). These results are in coincidence with the results of Sarker and Biawas (2002) who also obtained best regeneration at 0.5 mg/L

kinetin but explants source was immature embryos and combination was also different (0.5 mg/L BAP, 25 mg/L tyrosine). Shah et al. (2003) also found good response when they added kinetin in the regeneration medium along with 1.0 mg/L IAA and 2 mg/L BAP.

REFERENCES

Alizadeh H, Naghave MR, Omidi M, Saatian B (2004). Effect of plant growth regulators on direct shoot regeneration of wheat (*Triticum aestivum* L.). New direction for a diverse planet: Proceeding of the 4th International Crop Congress Brisbane, Australia, 26 Sep-1 Oct 2004.

Harlan JR (1981). The Early History of Wheat. In: L.T. Evants, W.J. Peacock, editors. Wheat Science Today and Tomorrow. Cambridge, UK: Cambridge University Press. pp. 1-9.

Mohanty A, Sarma NP, Tyagi KA (1999). *Agrobacterium*-mediated high frequency transformation of an alite *Indica* rice variety Pusa Basmati 1 and transmission of the transgenes to R2 progeny. Plant Sci. 147: 127-137.

Mooney PA, Goodwin PB (1991). Adherence of *Agrobacterium tumefaciens* to the cells of immature wheat embryos. Plant Cell Tissue Organ Cult. 25: 199-208.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15: 473-497.

Peters NR, Ackerman S, Davis EA (1999). A modular vector for *Agrobacterium* mediated transformation of wheat. Plant Mol. Biol. Rep. 17: 323-331.

Rashid H, Ghani RA, Chaudhry Z, Naqvi SMS, Quraishi A (2002). Effect of media, growth regulators and genotypes on callus induction and regeneration in wheat (*Triticum aestivum* L.). J. Biotechnol. 1(1): 49-54.

Sarker RH, Biawas A (2002). *In vitro* plantlet regeneration and *Agrobacterium* mediated genetic transformation of wheat (*Triticum aestivum* L.). Plant Tissue Cult. 12(2): 155-165.

Shah MI, Jabeen M, Hai I (2003). Invitro callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum aestivum* L.). VARLU-268. Pak. J. Bot. 35(2): 209-217.

Turhan H, Baser I (2004). Callus induction from mature embryo of winter wheat (*Triticum aestivum* L.). Asian J. Plant Sci. 3(1): 17-19.

Wang AS, Evans RA, Altendrof PR, Hanton JA, Doyle MC, Rosichan JL (2000). A mannose selection system for production of fertile transgenic maize plants from protoplasts. Plant Cell Rep. 19: 654-660.