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Full Length Research Paper

Effect of explant plant source and acetosyringone concentration on transformation efficiency of wheat cultivars

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Gene introduction into crop plants through genetic manipulation is a better alternative to conventional breeding for the improvement of stress tolerance. *Agrobacterium*-mediated transformation offers precise integration of genes into the genome with enhanced transgene stability. There are a number of factors which influence the rate of genetic transformation. The theme of this study is to exploit the explant source and acetosyringone concentration for the efficient development of *Agrobacterium*-mediated gene delivery system in wheat cultivars Inqilab 91 and Chakwal 97. Seedlings, mature embryos and calli used as explant sources for transformation in both the cultivars showed positive response. It was further observed that mature embryos produced maximum transformation efficiencies of 40.0 and 36.25% with 37.5 and 31.03% of regeneration frequencies of transgenic plants for Chakwal 97 and Inqilab 91, respectively. Seedlings produced 33.75 and 27.5% while calli produced 26.25 and 22.5% transformation efficiencies for both the cultivars. Acetosyringone concentration is also a limiting factor in transformation experiments especially in the case of cereals crop. Different concentrations of acetosyringone were used at the time of co-cultivation for optimization of the transformation protocol and maximum transformation efficiencies of 52.44 and 47.56% were obtained with 50 µM of acetosyringone from the cultivars Chakwal 97 and Inqilab 91, respectively.

Key words: Wheat, agrobacterium, transformation, explant, acetosyringone.

INTRODUCTION

In Pakistan, the major staple food is wheat which is grown over an area of more than eight million hectares, hence is the largest grown crop. It shares 3% in GDP (Gross Domestic Product) and 14.1% as a whole in agriculture (Ahmad, 2009). Plant breeders are providing considerable attention to wheat genetic improvement from the past few years to minimize losses due to pests and pathogens and also to improve the grain yield (Pingali and Rajaram, 1999). Progress in crops genetic improvement for stress resistance is limited due to poor understanding of the mechanism and availability of efficient techniques for selecting breeding resources for stress resistance (Bhatti and Chaozu, 2009).

Genetic transformation is a promising alternative to conventional breeding for the introduction of genes into plants (Xing et al., 2008). Due to the speedy development in the field of genetic engineering, it has offered a promising approach to improve stress tolerance (Ramanjulu and Bartels, 2002; Islam et al., 2007). *Agrobacterium*-mediated transformation facilitates the precise integration of genes into the plant genome (Shou et al., 2004; Karami et al., 2009), however, this system is affected by a number of factors including tissue culture conditions, acetosyringone concentration, explant source etc (Briza et al., 2008; Cho et al., 2008). The theme of this study is

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Abbreviations: As, Acetosyringone; **Kn,** kinetin; **IAA,** indole 3-acetic acid; **BAP,** 6-benzylaminopurine; **2iP,** 6-(gamma, gamma-dimethylallylamino) purine; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **GUS,** beta-glucuronidase; **X-Gluc,** 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid.

to exploit the explant source and acetosyringone concentration for the efficient development of *Agrobacterium*-mediated gene delivery system in local cultivars of wheat.

MATERIALS AND METHODS

Seedlings, embryos and calli of two wheat cv. Chakwal-97 and Inqilab-91 were used as explant sources in this study. MS (Murashige and Skoog, 1962) media supplemented with 3 mg/l 2,4-D was used for initiating callus from the explants. The cultures were incubated in growth room at 25±1°C for 20 days for proliferation. After 20 days, the calli were shifted to maintenance medium that is, MS medium containing 3 mg/l 2,4-D. 20 days old calli were used for transformation with *Agrobacterium* strain EHA101 containing binary vector pIG121Hm. Explants were co-cultivated with the *Agrobacterium* for 1 min and then blotted dry. Co-cultivation plates contained callus induction medium (CIM) + different concentrations of As (Acetosyringone) that is, 0.0, 50 and 100 µM. These plates were placed at 28°C in dark for 1 - 2 days. Then the explants were disinfected with 500 mg/l cefotaxime and were transferred to selection medium (MS media + 3 mg/l 2,4-D + 50 mg/l hygromycin and 500 mg/l cefotaxime) for 30 days. The transformed explants were shifted to regeneration media containing different growth regulators (2ip, IAA, BAP and Kn) and 50 mg/l hygromycin and 500 mg/l cefotaxime. Some of the explants were selected at random for GUS assay after 15 days of selection and were incubated in X-Gluc solution at 37°C for 2 - 3 days for confirmation of transformation.

RESULTS AND DISCUSSION

Callus induction

Calli were induced from seedlings and mature embryos of two wheat cultivars (Inqilab 91 and Chakwal 97) on MS medium with four different concentrations of 2,4-D that is, 2.0, 3.0, 4.0 and 5.0 mg/l. In addition, a control combination on MS medium without 2,4-D was also kept to compare the results. Highest percentage of 76.0 and 61.0 were achieved from Inqilab 91 and Chakwal 97 respectively with 3 mg/l of 2,4-D. No callus induction was recorded without 2,4-D (Table-1). Nasircilar et al. (2006) obtained best results of callus induction with 2.0 mg/l of 2,4-D in MS medium for different wheat cultivars. Haliloglu (2002) observed highest embryogenic callus formation from immature embryos of wheat on MS+B5 medium with 2.0 mg/l of 2,4-D. These results with slight difference in concentration of 2,4-D may be due to difference in genotypes because varied concentration of 2,4-D have been recorded for different genotypes by Arzani and Mirodjagh (1999). Shah et al. (2003) recorded best callus induction at 3.5 mg/l and good callus induction at 3.0 mg/l of 2,4-D. Malik et al. (2004) found 3.5 mg/l of 2,4-D concentration in LS medium as the most appropriate callus induction and proliferation from mature seeds in wheat cultivars. These results are in line with our study and 3.0 mg/l of 2,4-D was best for callus induction. Rahman et al. (2008) achieved best callus induction with 6.0 mg/l of 2,4-D from seeds and mature embryos which is quite in contrast with the present study.

Regeneration of calli

Regeneration of calli derived from seedlings and mature embryos of wheat was carried out with two different combinations of growth regulators in MS medium. Maximum regeneration of 75 and 71% was observed from the mature embryos derived calli of Inqilab 91 and Chakwal 97 respectively with BAP (1 mg/L) + Kinetin (0.5 mg/L) +2iP (0.5 mg/L). Rashid et al. (2002) achieved best regeneration with 0.1 mg/l of IAA and 0.5 mg/l of BAP in wheat. cv Rawal-87. Alizadeh et al. (2004) found 1 mg/l of BAP, 0.2 mg/l IAA and 0.2 mg/l of 2,4-D as a best combination for shoot regeneration from embryos and 0.2 mg/l 2,4-D and 2 mg/l BAP as a best combination for shoot regeneration in excised embryo explants. Shah et al. (2003) found 2 mg/l BAP and 1.0 mg/l IAA as best combination for plantlet regeneration and also found Kn good regeneration (Table 2).

Effect of explant source on transformation efficiency

Three different explant sources (seedlings, mature embryos and calli) were used for transformation in two wheat cultivars Chakwal 97 and Inqilab 91. All the explant sources used for transformation in both the cultivars showed positive response. It was observed that mature embryos produced maximum transformation efficiencies of 40.0 and 36.25% and maximum regeneration frequencies of 37.5 and 31.03 for Chakwal 97 and Inqilab 91 respectively. Seedlings produced 33.75 and 27.5% while calli produced 26.25 and 22.5% transformation efficiencies for both cultivars (Table 3). Type of explant was found to affect transformation efficiency (Lengliz et al., 2009). Sarker and Biswas (2002) tested four different explants for their transformation ability and obtained maximum transformation from immature embryos derived calli. Patnaik and Khurana (2003) used mature embryo derived calli of Triticum aestivum and T. durum for transformation through particle bombardment and obtained the transformation frequency of 7.7 and 10%, respectively. Mature embryos of T. aestivum cv. CPAN1676 and T. durum cv. PDW215 were co-cultivated with Agrobacterium tumefaciens LBA4404 (pBI101::Act1) and achieved transformation efficiency of 5.57% (Patnaik et al., 2006).

Effect of acetosyringone concentration on transformation efficiency

Different concentrations of acetosyringone (0, 50 and 100 µM) were used at the time of co-cultivation and in the co-cultivation plates. Maximum transformation efficiencies of 52.44 and 47.56% were obtained with 50 µM of acetosyringone from the cultivars Chakwal 97 and Inqilab 91 respectively (Table 4). These results are supported by the results of McCormac et al. (1998) who used 100 µM

Table 1. Percentage of calli derived from seedlings and mature embryos with different concentrations of 2,4-D.

Concentrations of 2,4-D (mg/l)	Inqilab 91		Chakwal 97	
	Seedlings	Mature embryos	Seedlings	Mature embryos
0.0	0.0	0.0	0.0	0.0
2.0	34	42	41	58
3.0	62	76	56	61
4.0	32	38	35	40
5.0	21	26	25	31

Values are percentage in round figures.

Table 2. Regeneration percentage of calli derived from seedling and mature embryos on regeneration medium with different combinations of growth regulators.

Variety	IAA (0.1 mg/L) + BAP (1 mg/L)		BAP (1 mg/L) + Kinetin (0.5 mg/L) + 2iP (0.5 mg/L)	
	C1	C2	C1	C2
Inqilab 91	46	52	60	75
Chakwal 97	49	55	57	71

C1 = Seedlings derived calli; C2 = mature embryos derived calli.

Table 3. Transformation efficiency of wheat cultivars using different explants at 50 µM acetosyringone.

ES	TNE	CSGA	GPC	PGA	TCSH	SEH	TE	PF	RFT
Chakwai 97									
E1	100	20	9	45	80	27	33.75	7	25.93
E2	100	20	13	65	80	32	40.0	12	37.5
E3	100	20	7	35	80	21	26.25	5	23.80
Inqilab 91									
E1	100	20	7	35	80	22	27.5	4	18.18
E2	100	20	11	55	80	29	36.25	9	31.03
E3	100	20	6	30	80	18	22.5	4	2222

ES = Explant source, E1 = seedlings, E2 = mature embryos, E3 = calli, TNE = total number of explants, CSGA = calli selected for GUS activity, PGA = percentage of GUS analysis, SEH = selected explants on hygromycin, GPC = GUS positive calli, PF = plantlet formation, TCSH = total calli for selection on hygromycin, TE = transformation efficiency (%), and RFT = regeneration frequency of transgenic plants.

Table 4. Transformation efficiency of wheat cultivars using mature embryos as explant source with different concentrations of acetosyringone.

AC	TNE	CSGA	GPC	PGA	TCSH	SEH	TE
Chakwal 97							
0.0	100	18	0	0.0	82	0	0.0
50.0	100	18	11	61.11	82	43	52.44
100.0	100	18	7	38.9	82	31	37.80
Inqilab 91							
0.0	100	18	0	0.0	82	0	0.0
50.0	100	18	9	50.0	82	39	47.56
100.0	100	18	6	33.33	82	27	32.93

AC = Acetosyringone concentration in Mm, TNE = total number of explants, CSGA = calli selected for GUS activity, PGA = percentage of GUS analysis, SEH = selected explants on hygromycin, GPC = GUS positive calli, TCSH = total calli for selection on hygromycin, and TE = transformation efficiency (%).

As and found that presence of As increase the efficiency of transformation. When they used 100 µM of As, transformation efficiency was 46.15% while in the absence of As, the efficiency was 6.66%. Ke et al. (2002) added 100 µM As to inoculation and co-cultivation media and observed development of GUS loci within the shoot and root structures. Amoah et al. (2001) added As to final concentration of 200 µM and obtained increased number of explants producing blue spots. The present study can further be improved by testing the impact of higher concentrations of As such as 150 and 200 µM on efficiency of transformation. The mature embryos after 15 days of selection were tested for transient GUS expression. In case of 0 µM of As, no GUS expression was observed. Maximum GUS expression was observed by using 50 µM of As.

In the present study an efficient transformation protocol was developed by exploiting explant source and acetosyringone concentration which will lead to the further improvement of wheat quality as well as yield globally.

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