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SCREENING COMMERCIAL WHEAT (*Triticum aestivum* L.) VARIETIES FOR *AGROBACTERIUM MEDIATED* TRANSFORMATION ABILITY

Nabeela Abid, Asma Maqbool* and Kauser A. Malik

Department of Biological Sciences, Forman Christian College, Lahore, 54600, Pakistan
***Corresponding author's e-mail: asmamaqbool@fccollege.edu.pk**

Wheat is staple food crop of many countries including Pakistan. It has a large number of cultivars and genotypes. All genotypes have different tissue culture response that includes callus induction, regeneration and transformation efficiency. For transgenic plant production it is crucial to know tissue culture efficiency of a selected variety. Therefore, in the present study mature embryos of thirteen elite wheat (*Triticum aestivum* L.) varieties were evaluated for tissue culture response and their amenability to transformation. Each variety responded differently for callogenesis, transient GUS (β-glucuronidase) expression and regeneration. The results for callus induction and transient GUS expression ranged from 30-100% and 13-100%, respectively whereas regeneration response was quite different in tested varieties that ranged from 0-44%. Good quality callus was observed in all varieties except Dhurabi-11, Lasani-08, Millat & Pak-81. Maximum transient GUS expression (100%) was found in Faisalabad-2008. Highest regeneration (44%) was noticed in Pak-81. Results indicated that three varieties VIII-83, Faisalabad-2008 and Aas-11 are suitable for transformation in comparison to others.
Keywords: wheat, tissue culture, callus, regeneration, transient *GUS* expression, *Agrobacterium-mediated* transformation

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important staple food and one of the dominant crops in temperate countries (Shewry, 2009; Zale *et al.,* 2009). It is grown on more than 240 million hectares, with 564.6 million tons production, an average of 2500 kg grain per hectare, larger than any other crop (Alam, 2001). In Pakistan wheat is grown on 36% of total crop area. World trade in wheat is greater than for all the other crops combined. It is the most favored staple food. Wheat provides more nourishment for humans than any other food source. It contains protein, minerals, vitamins and fats (lipids). A wheat-based meal is highly nutritious and is higher in fiber than a meat-based diet (Johnson *et al*., 1978; Sramkova *et al*., 2009).

A number of biotic and abiotic factors like drought stress, salt affected areas, pest, herbs, and diseases are involved in decreasing the per hectare yield of wheat. Breeders have developed Wheat varieties with higher yield and minimum losses due to biotic and abiotic stresses. In recent year's biotechnology is also playing important role in improving yield of many crops including wheat (Patnaik and Khurana, 2001).

Many reports have described various factors including explants source, age of explants, media composition and effect of different phytohormones influencing transformation efficiency in wheat by biolistic and Agrobacterium mediated transformation (Demeke *et al.,* 1999; Wei *et al*., 2000; Haliloglu and Baenzinger, 2003; Shewry and Jones, 2005; Raja *et al*., 2010). Wheat has a large number of cultivars and genotypes. All genotypes have different tissue culture response that includes callus induction, regeneration and transformation efficiency (Papenfus and Carman, 1987; Ozgen *et al*., 1998; Benkirane *et al*., 2000; Nasircilar *et al*., 2006; Yasmin *et al*., 2009). The success of genetic engineering in any crop is directly related to callogenesis and regeneration ability of the species (Jones, 2005; Vendruscolo *et al*., 2008). There is a need to screen the available wheat genotypes for tissue culture response. In the present study we compared 13 different winter wheat varieties for callus formation, regeneration and transient expression.

MATERIALS AND METHODS

***Explants*:** Mature seed's embryos of 13 winter wheat (*Triticum astivum* L.) varieties (AARI-11, Aas-11, Dhurabi-11, Faisalabad-2008, Lasani-08, Millat-11, Pak-81, Punjab-11, Sahar-2006, Shafaq, V-07096, V-08203 and VIII-83) were used. Seeds of wheat varieties for present study were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Seeds were sterilized with 70% ethanol for 1-2 min, 50% Clorox bleach for 20 min followed by 4-5 washings with autoclaved distilled water and washing was given by continuous shaking. Sterilized seeds were soaked in autoclaved distilled water for 48 hours.

***Callus induction*:** Mature embryos were excised from seeds and shifted on MS media (Murashige and Skoog, 1962) supplemented with 3mg/l 2,4-D for callus induction. The cultures were incubated in dark culture room at 25±1°C for one week.

***Agrobacterium strain and plasmid*:** *Agrobacterium tumefaciens* strain *AgL1* harboring a binary plasmid pGA482 having GUS (uidA) gene and *nptII* (neomycin phophotransferase II) gene obtained from National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad was used for transformation of wheat calli (2-3 mm in diameter).

Single colony of *Agrobacterium* strain was grown in 10ml LB (Bacto tryptone 10 g/l + Bacto yeast extract 5 g/l + NaCl 10 g/l) medium (Bertani *et al*., 1951) at 120 rpm and 28^{o}C on a rotary shaker until OD (600) of 0.7-0.8 was achieved. The cells were further grown for 2-3h after adding 400µM Acetosyringone under same conditions. The grown culture was used for further experimentation.

***Inoculation and co-cultivation*:** For inoculation, calli were placed in the center of the plate and *Agrobacterium* culture was gently poured on the calli and left for 15-20 minutes. Excessive culture was removed by dragging calli on medium and shifted on co-cultivation medium (MS medium having 2mg/l 2,4-D and 400uM Acetosyrangone). The samples were incubated in dark at 25±1°C for further 2-3 days.

***Suppression of Agrobacterium overgrowth and plant regeneration*:** After 3 days of co-cultivation 15% calli of each variety were used for GUS assay and the remaining 85% were transferred to callus induction medium containing 160 mg/l of the antibiotic Timentin. Callus was maintained on callus induction medium for 3-4 weeks, after which they were transferred to regeneration medium (MS+1mg/l Kinetin).

***Assay for GUS activity*:** Histochemical GUS assay was conducted according to the method described by Jefferson and co-workers (1987. GUS expression was determined after two days of inoculation. Inoculated calli were incubated overnight at 37°C in buffer containing 1 mM X-Gluc, 100 mM sodium phosphate buffer pH 7.0, 0.5 mM potassium ferrocyanide and 0.1% (v/v) Triton X-100.

RESULTS AND DISCUSSION

***Callus formation*:** Response of different wheat varieties for callus induction was not drastically different. Hundred percent true callus formations were observed in nine varieties (AARI-11, Aas-11, Faisalabad-2008, Punjab-11, Sahar -2006, Shafaq-2006 V-07029, V-08203 & VIII-83). True calli have solid and rigged structure on the surface which is the indication of embryogenic calli. Similar observations were reported by (Satyavathi *et al*., 2004) compact and nodular structures on the surface of callus are the characteristic of embryogenic callus.

In three varieties Lasani-08, Dhurabi-11 and Millat-11 the response was 85%, 70% and 60%, respectively. The poor (30%) response of callus induction was found only in Pak-81. The appearance and growth rate of callus was different in each variety (Table 1). With respect to callus the varieties can be divided into three categories. True, good quality (embryogenic), healthy and larger calli were formed by AARI-11, Aas-11, Sahar -2006, V-07096, Faisalabad-2008, V-08203 and V-III-83. The callus induced by Lasani-08, Pak-81, Shafaq-2006 and Punjab-11 was also good but smaller in size (Fig. 1). With respect to callogenesis two varieties (Dhurabi-11 and Pak-81) are not good as Dhurabi-11 formed 70% true & 30% false calli while in Pak-81, 70% of the calli were bad and the rate of growth was very slow. False/bad calli were fleshy and whitish with shoots which is indication of non embryogenic calli as reported by Munazir *et al.* (2010) and Rashid *et al.* (2009). Non- embryogenic callus was fleshy and whitish in color along with shoots. These observations indicated the genotypic differences among wheat varieties. Several researchers have reported difference in tissue culture response among cultivars of bread wheat (Caswell *et al*., 2000; Przetakiewicz *et al*., 2003) and durum wheat (Bommineni and Jauhar, 1996;

Table 1. Response of callus formation in different wheat varieties from mature embryo

Name of Varieties	No of Embryos on CIM	% of calli formed	Quality of callus	% of True Callus
AARI-11	200	100%	Good & Healthy	100%
Aas-11	200	100%	Good & Healthy	100%
Dhurabi	200	100%	70% Good & 30% False/ Bad	70%
Faisalabad-2008	200	100%	Good & Healthy	100%
Lasani-08	200	85%	Good & small size	85%
Millat-11	200	60%	Good & Healthy	60%
Pak- 81	200	100%	Small size and callus formation was very slow. 70% false	30%
Punjab-11	200	100%	Good ,Healthy & smaller size	100%
Sahar -2006	200	100%	Good & Healthy	100%
Shafaq-2006	200	100%	Good & small size	100%
V-07096	200	100%	Good & Healthy	100%
V-08203	200	100%	Good & Healthy	100%
V-III-83	200	100%	Good & Healthy	100%

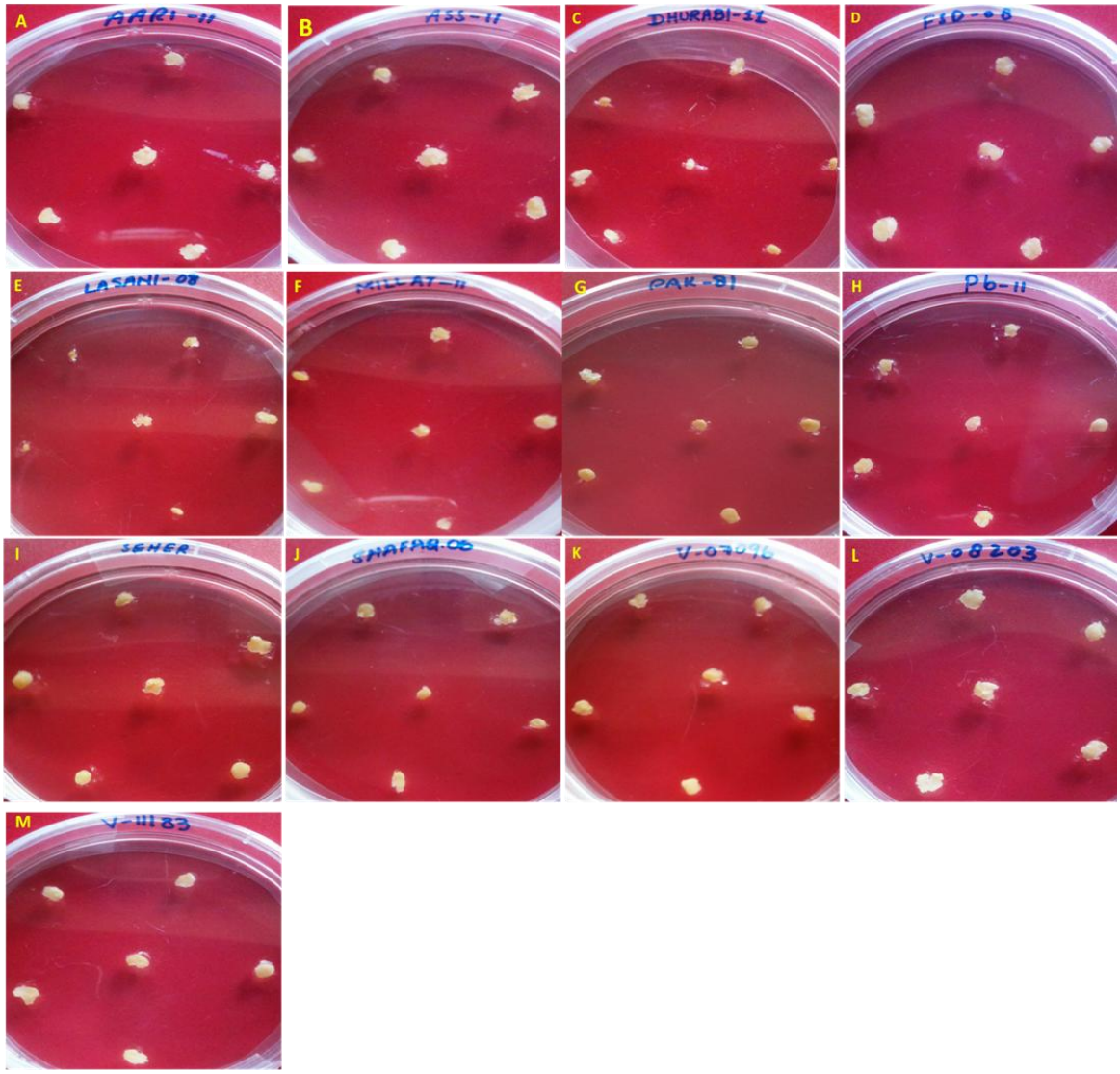

Figure 1. Callogenesis in different wheat varities.

A- ARRI 11, B- Aas-11, C- Dharabi-11, D- Faisalabad-08, E- Lasani-08, F- Millat-11, G- Pak-81, H- Punjab-11, I- Saher-06, J- Shafaq-2006, K- V-07096, L- V-08203, M- V-11183

Benkirane *et al*., 2000; Gonz´alez *et al*., 2001; Nasircilar *et al*., 2006). Frequency of callus induction in the present study varies from 30-100% that is quiet high. Similar results were reported by Khalid *et al.* (2013 which shows 27%-90% frequency of callus induction among wheat cultivars. In contrast to present work Nasircilar *et al*. (2006) reported very low callus induction frequency (25.6 % to 57.6 %) in different cultivars of *Triticum aestivum* and *T. durum*. Our results suggested that variable response of cultivars to tissue culture was due to genotype as postulated by Yasmin *et al*. (2009). These results are similar with those of Hassan *et al*. (2009), Shah *et al*. (2009) and Kilinc (2004) who suggested that callus induction depends upon genotype of wheat. Generally, the poor response of mature embryos for callogenesis has been reported in the literature (Ozen *et al*., 1996; Rahman *et al*., 2008). Tissue culture of wheat depends upon genotype of wheat (Mahmood *et al.,* 2012).

***Transient GUS expression*:** GUS expression was observed in calli of all varieties. The frequency of GUS expression was found to be variety dependent and ranged from 13-100%. Some varieties showed better result as compared to others. 100% GUS +ve result was observed in Faisalabad-2008, 87% in AARI-11 & Punjab-11, 70% in Sehar-2006, 40% GUS expression in the form of dots were observed in Dharabi-11, Lasani-2008 and Shafaq-2006, 27% at the edges of calli in AQS-11, Pak-81, V-07096 & V-III-83, 13% in

Figure 2. Transient GUS expression of wheat varieties after three days of co-cultivation

C- non transformed callus from different varieties, 1- Sehar-2006, 2- AQS-11, 3- AARI-11, 4- Punjab-11, 5- Faisalabad-2008, 6- Lassani-08, 7- Millat-11, 8- Shafaq-06, 9- Pak-81, 10- Dharabi-11, - V111-83, 12- V-07096, 13- V-08203

Millat-11 & V-08203 (Fig. 2). Similar type of GUS expression pattern has been reported in rice (Li *et al.*, 1993), and Sweet potato (Prakash and Vardarajan, 1992). Faisalabad-2008 showed better result than all the other varieties.

***Regeneration*:** Our results showed that the regeneration efficiency ranged from 08-44% in 13 wheat cultivars. Millat-11 and Shafaq-2006 are not good for regeneration. In literature it is well documented that regeneration is highly genotype dependent (Wei *et al.,* 2003; Haliloglu and Baenziger, 2005; Aydin *et al.,* 2011). Our results are in accordance with Yu *et al*. (2008) who reported 17.8 to 36.8% regeneration rate from mature embryos among different wheat genotypes. Ozgen *et al*. (1996) reported an average of 70.4% regeneration capacity for mature embryo culture. However Delporte *et al.* (2001) reported 70.20% regeneration capacities. Significant differences observed in regeneration among wheat cultivars by Khalid *et al.* (2013) who reported that regeneration depends upon genotype of wheat so that each genotype behaved differently at different levels of growth regulators. The results indicated that callus induction, transformation efficiency and regeneration efficiency are not directly linked (Fig. 3). Maximum regeneration (44%) was observed in 'Pak- 81' while the callus response was poor (30%) and efficiency of transformation was average (27%). Shafaq-2006 had maximum callus induction and transformation efficiency of 100% and 40%, respectively but no (0%) regeneration responses (Fig. 4). Similar result has been reported by Galovic *et al*. (2010) in his study on wheat mature embryo-derived transformation. In comparison to mature embryo regeneration from immature embryos is considerably higher (Redway *et al.,* 1990) but immature embryos are not available throughout the year. In contrast, mature embryos could be available at any time (Ozgen *et al*., 1996; Ozgen *et al.*, 1998).

***Conclusions*:** Based on the results it can be concluded that three varieties (V-III-83, Faisalabad-2008 and AQS-11) were comparatively better in tissue culture response and might be used for transformation to get good results.

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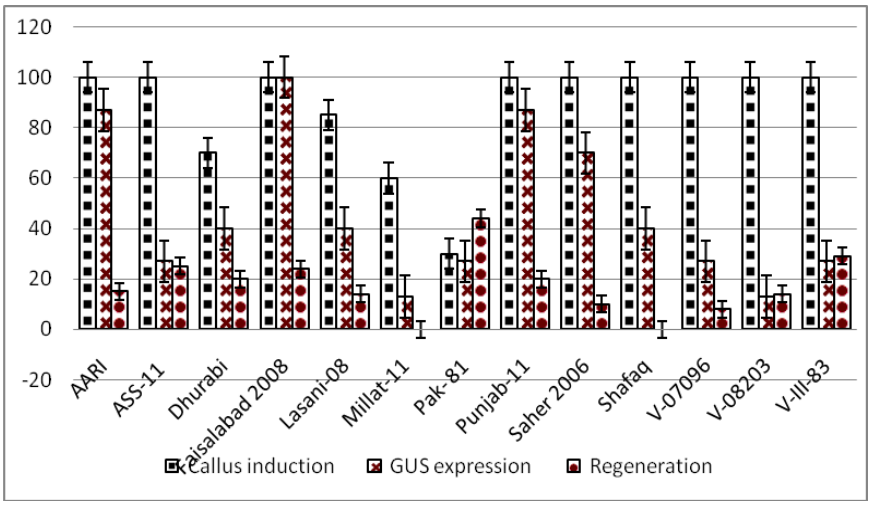

Figure 3. Comparison of callus induction, transient *GUS* expression and regeneration in different wheat varieties.

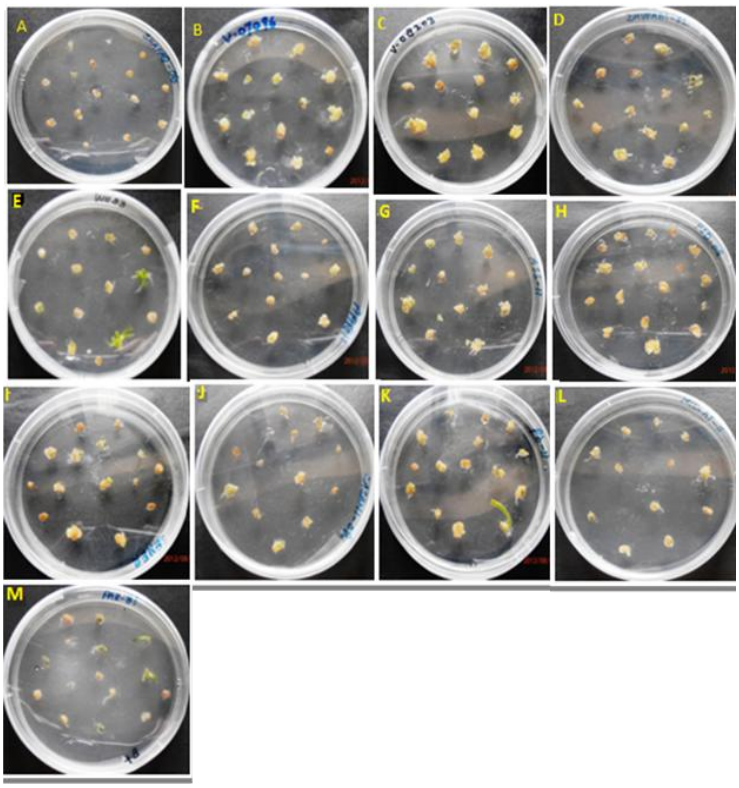

Figure 4. Regeneration responses in different wheat varieties.
A- Shafaq-06, B- V-07096, C- V-08203, D- Dhorabi-11, E- V-III-83, F- AARI-11, G- Aas-11, H- Faisalabad-08, I- Sehar-06, J- Lasani-08, K- Punjab-11, L- Millat-11, M- Pak-81

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