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Review Article

Genetic transformation of wheat–Present status and future potential

Mamrutha HM[1]*, Rakesh Kumar[2], Karnam Venkatesh[1], Pradeep Sharma[1], Raj Kumar[1], Vinod Tiwari[1] and Indu Sharma[1]

[1]*ICAR-Indian Institute of Wheat & Barley Research, Karnal-132001, Haryana, India*
[2]*University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra-136119, Haryana, India*

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****Corresponding author***

Email: *mamruthamadhu@gmail.com*
Tel.: *08053243212*

Abstract

Wheat is the major staple food crop of the world immediately after rice. Since green revolution, its production has increased with the introduction of input responsive, semi dwarf, high yielding varieties. In the recent years, conventional breeding helped in sustaining the yield levels but failed to provide a significant yield jump. Under such circumstance genetic transformation could serve as a useful tool for transferring target gene of interest for further crop improvement. Transformation in wheat has been least successful because of low transformation efficiency and genotype dependency. During last decade, tremendous efforts has been made using either biolistic particle or *Agrobacterium*-mediated transformation with different explant types to transfer several marker genes, biotic and abiotic stress tolerance genes into wheat. This review presents different techniques applied in wheat transformation with a view to encourage further long-term wheat improvement research by genetic engineering approaches.

Keywords: Wheat, tissue culture, genetic transformation, transgenics, *Agrobacterium*

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple food crops of the world which is grown in more than 17% of the cultivable land and is consumed by ~40% of the global population (Goyal and Prasad, 2010; Peng *et al.,* 2011). Wheat fulfils 21% of the total calorie and 20% of the protein requirements of more than 4.5 billion population in developing countries (Braun *et al.,* 2010). In India, in the year 2013-14 wheat occupied about 30 million hectare area with the production of 95.91 million tonnes (DES, 2014). India is a major contributor to the world wheat production after China has witnessed a tremendous increase in production during the last four decades. By having green revolution through development and utilisation of input-responsive, high yielding disease resistant and adoption of environment friendly cultivation practices such as conservation tillage/agriculture. Despite this generally increasing trend, wheat production in India is being constantly challenged by many threats as reported world-wide (Xia *et al.,* 2012) in the form of climatic changes leading to temperature extremes, weather changes, erratic precipitation and changes in pest dynamics. Overall changes in the micro-environments are expected to be more severe in the coming years coupled with challenges due to decrease in arable land caused by increasing urbanization, land degradation and limited water for irrigation (Anonymous, 2011). Present wheat improvement methods through conventional breeding in India are being confronted by slow progress in the genetic improvement of yield potential and stability. Although conventional breeding programmes have led to continuous improvements in some of the economically important traits, emergence of new threats and climatic challenges calls for a faster action towards development of varieties resistant to emerging pests and diseases, and abiotic stresses.

With the advent of rDNA technique and transformation methods, a new way of incorporating defined genetic changes into plants is quite possible. The continuous development of *Agrobacterium*-based gene transfer systems and biolistics methods for improving its efficiency and applicability to many crops is rapidly replacing other methods for generation

of transgenic plants, but between these two methods *Agrobacterium* method is more robust compared to the biolistics approach. Nevertheless, tissue culture techniques have played a major role in transferring gene of interest and transgenic plant recovery. Davis and Reznikov (1992) documented in their work the classical example which has been used in a range of protoplast, microspore, tissue and organ culture protocols. Despite several disputes, field trials of transgenic plants have recently become much more common. The worldwide area under GM crops plantation during the 15 years from 1996 to 2010 exceeded, for the first time, up to 1 billion hectares. Engineered traits such as herbicide tolerance and insect resistance have resulted in a significant reduction in herbicide and insecticide use (James, 2010). Therefore, genetic engineering based crop improvement would likely be considered as a method of choice. Successful transgenic plant recovery involves well-established transformation protocol. Many plant species have been genetically modified, either by *Agrobacterium* dependent or independent method. Therefore, in near future, tissue culture dependent inter species biotic/abiotic gene transfer process will hold a method of choice for efficient gene transfer and transgenic recovery (Hinchee *et al.,*1994) for increasing wheat yield.

2. Updates of transformation in wheat

2.1. Selection of wheat genotypes: Triticum aestivum is the most widely utilized species for tissue culture studies. Within this species, the spring wheat cv. Bobwhite accounts for >25% of the data reported until now. The other cultivars tested includes Chinese Spring (Langridge *et al.,* 1992); Kedong58, Rascaland Scamp (McCormac *et al.,* 1998); Lona (Uze *et al.,* 2000); Baldus (Amoah *et al.,* 2001); Fielder (Weir *et al.,* 2001); Florida and Cadenza (Wu *et al.,* 2003); Vesna (Mitic *et al.,* 2004); Veery5 (Khanna and Daggard 2003; Hu *et al.*, 2003), Turbo (Hess *et al.,* 1990) and Millewa (Mooney *et al.,* 1991) etc. However, a limited amount of work has been done in other species of wheat like *T. dicoccum* (Khuranna *et al.,* 2002; Chugh and Khurana, 2003), *T. durum* (Patnaik *et al.,* 2006) or *T. turgidum* (Wu *et al.,* 2008; Wu *et al.,* 2009; He *et al.*, 2010). As earlier work revealed that regeneration ability in tissue culture is largely depending on cultivar used, there is imperative need to standardize the tissue culture protocol for each provincial cultivar across the world.

2.2. Type of explants: The immature embryos of 11-16 d post anthesis with ~0.8-1.5 mm in size were the most widely used explants in most of the *Agrobacterium*-mediated transformation and this tissue has highest regeneration capacity compared to other explants (Jones, 2005). The first successful wheat transformation was done by Cheng *et al.* (1997) using immature embryos of Bobwhite and transformed with *nptII* and *gus* gene yielded T_1 lines with Mendelian inheritance. This success paved the way for other researchers working in cereals especially in wheat by using immature embryos of Veery5 (Khanna and Daggard 2003), Xinchun9, PM97034, Yangmai10, Sakha206 (Clemente and Mitra 2005), Yangmai10 (Li *et al.,* 2005), Vesna (Mitic *et al.,* 2004) cultivars in different experiments for transferring the gene of interest. Again immature embryos of Bobwhite were used to transform with *EPSPS* gene, an herbicide-resistant gene (Hu *et al.,* 2003, Zhou *et al.,* 2003). Apart from bread wheat, immature embryos of durum wheat were also used as explants by Wu *et al.* (2008) and obtained transformation efficiency between 0.6 and 9.7%. In durum wheat, immature embryos of Stewart along with acetosyringone concentration from 200 to 400 µM have improved the transformation efficiency (He *et al.,* 2010).

Compared to immature embryos, the mature embryos are regarded as convenient explants for wheat genetic transformation, as they are easily available throughout the year. But the regeneration frequency from mature embryos of many wheat cultivars is very low, which limits its use in wheat transformation. Three different wheat cultivars namely Lunxuan987, Yumai66 and Bobwhite were transformed using *Agrobacterium* C58C1 carrying pUbi GNGUS vector and the putative transformants were confirmed by PCR and southern blot with a transformation efficiency of 0.12-1.79%. Mature embryos were also successfully used as explants in different *T. durum* transformation systems (Khurana *et al.,* 2002, Vishnudasan *et al.,* 2005, Ding *et al.,* 2009).

Anthers are being used as explants mainly under double haploid (DH) production program. Barley *HVA1* gene was successfully transformed using anther culture-derived haploid embryos of wheat × maize system. Transgenic haploid plants were doubled by colchicine treatment and were confirmed for expression of *HVA1* by RT-PCR and western blot. These transgenic plants showed better drought tolerance compared to non transgenic plants under controlled condition (Chauhan and Khurana, 2011).

Apical meristem of vegetative/reproductive organ is used as explants. The β-1,*3-glucanase* gene was transformed to wheat and got resistance to powdery mildew using stem tips as explants (Zhao *et al.,* 2006). Bud tips were used to puncture and transform *agrobacterium* culture and got transformation efficiency of 33% (Supartana *et al.,* 2006). Floral organs are very frequently used for direct transformation using *in planta* technique and are independent of tissue culture activities (Zale *et al.,* 2009).

2.3 Methods of wheat transformation: In biolistic or micro-projectile bombardment method, gold or other particles coated with plasmid suspension carrying target genes are shot into wheat cells. Currently, Bio-Rad's PDS-1000/He gene delivery system is more commonly used. There are several factors influencing gene delivery into target cell, which includes biolistic transformation parameters, such as, (a) rupture pressure, bombarding distance, DNA purity

and concentration, $CaCl_2$ concentration and spermidine concentration, and (b) biological parameters such as explant types and physiological status, culture conditions before and after bombardment, screening procedures, and regeneration rates of putative transgenic plants. Biolistic wheat transformation has been reported using different genes of interest (Kasirajan *et al.,* 2013).

Agrobacterium-mediated *in vitro* wheat transformation method has several advantages over other approaches, as it transfers large segments of DNA with minimal rearrangement, fewer copy gene insertion, higher efficiency and minimal cost. The *Agrobacterium tumefaciens* carries Ti plasmid, while another strain *A. rhizogenes* carries Ri plasmid, and a transfer DNA (T-DNA) in these two types of plasmids can be transferred into plant cells. The *Agrobacterium*-mediated transformation is very common in dicot plants as they produce natural inducers, but later several modifications has been made to use this technique successfully in monocots plants as well. First, stable transformation of wheat using immature embryos as explants by *Agrobacterium*-mediated transformation, demonstrated the successful transmission of the transgene to the next generation in a span of 3 months (Cheng *et al.,* 1997). There are several studies using different explants have followed *Agrobacterium* transformation and got transgenics. The *Agrobacterium* culture can also be directly used on plant reproductive organs for direct transformation and can avoid tissue culture process (Amoah *et al.,* 2001). But, there is limited success of this technique in wheat. In near future, *Agrobacterium* will be employed as a reliable, efficient and economical vector for the introduction of foreign genes into wheat by various laboratories throughout the world. Large reports of *Agrobacterium*-mediated transformation are discussed later part.

2.4 Agrobacterium strains and binary vectors: In wheat transformation, according to the published reports, the *Agrobacterium* LBA 4404 (more than 44%) is the most widely utilized strain followed by C58C1 (24%) and AGL 1 (24%). Other strains like A 281, GV 3101, ABI, EHA 101, EHA 105, AGL 0, M-21 have also been tested but reports are scanty (Trick and Finer, 1997; McCormac et al. 1998; Peters *et al.,* 1999; Weir *et al.,* 2001; Cheng *et al.,* 2003). Two *A. rhizogenes* strains, *viz*., LBA 9402 and Ar2626 have also been used. AGL0 and AGL1 have been engineered to contain the hyper virulent Ti plasmid, pTiBo542 harbouring additional *vir* genes originating from the *Agrobacterium* A 281 which in its oncogenic form possesses a broad host range and induces large, rapidly growing tumours. Hence, these strains do provide higher transformation efficiency (Jin *et al.,* 1987; Khanna and Daggard, 2003; Wu *et al.,* 2003; Mitic *et al.,* 2004). But there are some studies in wheat where good transformation was achieved without hyper virulent plasmid (Xia *et al.,* 1999; Weir *et al.,* 2001). The most common *Agrobacterium* strains used in wheat transformation below to hyper virulent group is the disarmed plasmid pTiBo542 from *A. tumefaciens* A 281 harbouring additional virulence genes *vir* B, C and G which confers the hyper virulence (Komari *et al.,* 1990). Two different constructs have been widely employed to carry extra *vir* region first, using the helper plasmid pAL155 which is a derivative of pSoup modified by the addition of *vir* G (Amoah *et al.,* 2001; Ke *et al.,* 2002; Wu *et al.,* 2008) and second, using different plasmids as pAL154, pAL186 or pTOK233 carrying 15 kb Komari fragment containing a set of *vir* B, C and G (Amoah *et al.,* 2001; Wu *et al.,* 2003; Mitic *et al.,* 2004; Przetakiewicz *et al.,* 2004; Wu *et al.,* 2008; Wu *et al.,* 2009; He *et al.,* 2010).

2.5 Promoters: Constitutive promoters like *CaMV35S* (cauliflower mosaic virus) and *ubi1* (maize ubiquitin) have been mostly utilized for transformation as these were found to be more compatible with wheat system and gave successful expression of inserted genes. Other promoters such as *act1* (rice actin promoter), *nos* (nopaline synthase) and *ScBV* (sugarcane bacilliform virus) have also been used (Hu *et al.,* 2003).

3. Agrobacterium-mediated transformation methodologies in wheat

3.1 Explants sterilization and pre-treatment: Maintaining asceptic conditions is the most important prerequisite in tissue culture. Researchers do have standardized different sterilization steps for different explants types, e.g., for immature embryo, 70% ethanol for 1-2 min followed by 20% bleach for 15 min (Li *et al.,* 2011). For mature embryo, 70% ethanol for 5 min and then treatment with 10% domestos (Amoah *et al.,* 2001).

3.2 Pre-culture: Along with MS media different concentration of synthetic auxins mainly 2,4-D (2,4-dichlorophenoxyacetic acid), 2.0mg l^{-1}; Picloram (4-amino-3, 5, 6-trichloropicolinic acid), 1.5 mg l^{-1} and Dicamaba (3,6-Dichloro-2-methoxybenzoic acid), 1.5 mg l^{-1} has increased the callus induction efficiency in Indian wheat varieties (Mamrutha *et al.,* 2012). The duration for pre-culturing varies from 4 to 5 days (Amoah *et al*., 2001; Ding *et al.,* 2009).

3.3 Inoculation: Several factors such as time, temperature, media strength, *Agrobacterium* cell density and inducers of stable transformation such as acetosyringone, sugars, auxins or surfactants influence transformation efficiency. Inoculation of *Agrobacterium* suspension can be done for quite variable period of time (15 min to 12 h). There is a general consensus that the optimal time of inoculation for T-DNA delivery is around 3 h (Jones *et al.,* 2005; Wu *et al.,* 2008; Ding *et al.,* 2009) and optimum temperature is 25°C (Supartana *et al.,* 2006). Surfactants, like pluronic acid F68 (0.02%; Cheng *et al.,* 1997) and Silwet L-77 (0.01%; Jones *et al.,* 2005) are known to increase the transformation efficiency by favouring the *A. tumefaciens* attachment during inoculation. But there are also some contrasting reports that presence of surfactants has no effect on

transformation efficiency (Haliloglu and Baenziger, 2003). The presence of acetosyringone in the inoculation medium is very crucial (200 µM concentration) and it clearly increases the transformation efficiency (Wu *et al.,* 2003, He *et al.,* 2010). The addition of some sugars, like maltose or glucose to the inoculation medium was essential to achieve efficient T-DNA delivery (Cheng *et al.,* 1997; Wu *et al.,* 2003). *Agrobacterium* optical cell density at 600 nm close to ≥1.0 (Jones *et al.,* 2005; Amoah *et al.,* 2001) during inoculation were reported to be crucial for efficient transformation.

3.4 Co-cultivation: Short periods of 2-3 days have been proposed as optimum for high transformation efficiency (Cheng *et al.,* 1997; Amoah *et al.,* 2001; Wu *et al.,* 2003; Ding *et al.,* 2009). Presence of acetosyringone and maintaining a temperature of 21^{0}C is crucial for co-culture (Wu *et al.,* 2003). Acetosyringone is found to induce the virulence genes present in Ti plasmid of *Agrobacterium tumefaciens* and helps in T-DNA transfer (Cheng *et al.,* 1997; Bi *et al.,* 2006). In dicots the natural host plants of agrobacterium acetosyringone is produced by crown gall cells, but in monocots an optimum(200µm) concentration of acetosyringone was externally supplied in inoculation and cocultivation media(Cheng *et al.,* 1997; Cheng *et al.,* 2003; Chugh and Khurana, 2003; Bi *et al.,* 2006; Patnaik *et al.,* 2006; Wang *et al.,* 2009; Chugh *et al.,* 2012). However some researchers used 100 µM (McCormac *et al.,* 1998, Ke *et al.,* 2002), 250 µM (Khanna and Daggard, 2003), 400 µM (He *et al.,* 2010; Abid *et al.,* 2014) and 500 µM (Hensel *et al.,* 2009) concentration of acetosyringone in their studies.

3.5 Control of Agrobacterium, regeneration and selection: After co-culturing, maintaining optimum load of *Agrobacterium* in plant cells by avoiding overgrowth is important. Hence, antibiotics like timentin or carbenicillins are commonly used but other compounds such as cefatoxin, cefotaxime, ticarcillium and vancomycin have also been used. Along with this, the selection of the transformed plants is done by using marker gene on T-DNA to kill or compromise the growth of untransformed cells. Hence normally three types of selection agents, first for selecting only *Agrobacterium* (rifampicin), second for restricting the over growth of *Agrobacterium* cells (cefatoxime/ticarcillium/vancomycin/ cefatoxin) and third for selecting only transformed tissues depending on T-DNA construct (kanamycin) are generally used in regeneration media.

3.6 Selection of transformed tissues/transgenic plants: Two types of markers, viz., scorable and selectable are primarily used for selection of putative transgenics. Scorable markers are the ones which are visible mainly by histochemical and enzyme assays. A selectable marker includes the selection of transgenics on the basis of tolerance to specific antibiotics. The most commonly used scorable markers in wheat transformation includes GUS enzyme which hydrolyzes glucuronide compounds to gives reaction products that can be quantified using a spectrophotometer (Jefferson *et al.* 1987). Due to the simple histochemical detection procedure, the *gus* reporter gene system is extremely useful for optimisation of parameters for genetic transformation. However, one of the major limitations of *gus* reporter gene system is the destructive nature of its assay. The other scorable reporter gene like green fluorescent protein (*GFP*) from jelly fish *Aqueo reavictoria* (Pang *et al.* 1996; McCormac *et al.* 1998) and *luciferase* gene from fire fly *Photinus pyranus* (Lonsdale *et al.* 1998; Harvey *et al.*1999) have also been successfully utilized in wheat transformation.

Selectable marker uses the expression of an enzyme that confers resistance to cytotoxic substance mainly an antibiotic/ herbicide. Most commonly used selectable markers in wheat includes *bar*, *EPSPS, hpt, npt, CP4, MPI,* etc. The *bar* gene encodes for phosphinothricin acetyl transferase and is resistance to biolophos isolated from *Streptomyces* spp. The *neomycin phosphate transferase* (*nptII*) gene from bacteria gives resistance to amino glycoside antibiotics. EPSPS codes for 5-enopyruvylshikimate-3-phophate synthase, a critical enzyme in aromatic amino acid biosynthesis. CP4 codes for enolpyruvyl shikimate phosphate synthase (Zhou *et al.* 1995). The h*ygromycin phosphor transferase* (*hpt*) gene gives resistance against amino cyclitol antibiotic hygromycin B (Ortiz *et al.* 1996). Recently, several other selectable marker genes like m*annose 6-phosphateisomerase* (*man*A), *cyanide hydratase* (*Cah*) gene, *aceto lactate synthase* (*ALS*) (Ogawa *et al.,* 2008). *AtMYB*12 (Gao *et al.* 2011) have also been used. *pmi* (phospho mannose isomerase) which is considered as a bio-safety marker, has been validated in wheat (Stoykova and Stoeva-Popova, 2011).

3.7 Root induction: Half-strength MS salts without any growth regulators have been commonly used for root induction of regenerated plantlets in wheat (Przetakiewicz *et al.,* 2003; Bi *et al.,* 2006; Binka *et al.,* 2012).

3.8 Hardening of transgenic plants: Once the putative positive transgenics are confirmed after growing on proper shooting and rooting media with selection agents, then the seedlings are slowly hardened to plant in open field conditions. For this the plantlets are transferred to paper cups containing ready-mix potting mixture like pearlite/vermiculite and were grown in growth chamber with polythene cover over them so that direct light effect is avoided. Slowly the plants are shifted to glass house conditions where a direct exposure to sunlight is avoided. Later, the hardened plants are transferred to open field conditions, where further characterization and screening for specific traits are carried out.

4. Updates on wheat transgenic research

The wheat transformation has been attempted worldwide in different species of *Triticum* using different explants and binary vectors. The different promoters and genes involved in biotic and abiotic stress tolerance were evaluated and provided some leads in transformation. An update of worldwide transgenic work in different wheat genotypes are discussed in Table 1.

Table 1. Current status of *Triticum* transformation using *Agrobacterium* system

Species/genotype	Explant	*Agrobacterium* strain	Binary vector	Promoter/gene	Transformation efficiency (%)	Reference
T. aestivum						
Turbo	SPK	C58C1	pGV3850::1103neo	*Nos:nptII*	1-2.6	Hess *et al.*, 1990
Millewa	IE				1- 2	Mooney *et al.*, 1991
Chinese spring	SPK	LBA4404, A281, C58C1, GV3101	pB1121, pPCV6NFHygro	*CaMV35S:gus, Nos:nptII, CaMV35S:hpt*	0.8-4.7	Langridge *et al.*, 1992
Ganmai8	YE	C58C1	pGV3850::1103neo	*Nos:nptII, Nos:nos*	NR	Yao *et al.*, 1993
Bobwhite	IE, PCIE, IEdC	C58(ABI)	pMON18365	*CaMV35S:gus, CaMV35S:nptII*	0.14-4.3	Cheng *et al.*, 1997
Bobwhite	MSD	EHA105	pIG121Hm	*CaMV35S:gus, Nos:nptII, CaMV35S:hpt*	NR	Trick and Finer, 1997
Rascal, Scamp, Kedong58	IEdC	EHA101, LBA4404, LBA9402, Ar2626	pBECKS.red, pBECKS. sgfp-S65T, pBECKS. GUSintron	*CaMV35S:Lc/C1, CaMV35S:gfp, CaMV35S:gus, Nos:nptII*	NR	McCormac et al. 1998
Chinese spring	MSdC, IEdC	GV3101	pMVTBP, pNFHK1, p35SGUSINT	*CaMV35S:gus, CaMV35S:nptII*	1.2-2.2	Peters *et al.*, 1999
Chinese (several)	IE, IEdC	AGL1	pUNN2	*Ubi1:nptII*	3.7-5.9	Xia *et al.*, 1999
Bobwhite, Lona	PCIE	LBA4404, EHA105, C58C1, LBA9402	pBin9UG	*Ubi1:gus*	NR	Uze *et al.*, 2000
Baldus	21 days INFdC	AGL1	pAL154/pAL156, pAL155/ pAL156, pSoup/pAL186	*Ubi1:gus, Ubi1:bar*	NR	Amoah *et al.*, 2001
Fielder	PCIE	AGL0	pTO134	*CaMV35S:gfp, CaMV35S:bar*	1.8	Weir *et al.*, 2001
Nongda146	IE, PCIE	AGL1	pAL155/pAL156	*Ubi:bar, Ubi:gus*	NR	Ke *et al.*, 2002
Sourav, Gourav, Kanchan, Protiva	IE, ME, IEdC, MEdC	LBA4404, EHA105	pBI121, pCAMBIA1301	*CaMV35S:gus, Nos:nptII*	NR	Sarker and Biswas, 2002
BAU146, BAU170	IE, IEdC	AGL1,EHA105, LBA4404	pCAMBIA3301, pBTAaB	*Ubi:bar, Ubi:gus*	NR	Wang *et al.*, 2002
Bobwhite	PCIE, IEdC	C58(ABI)	pMON18365	*CaMV35S:gus, CaMV35S:nptII, CaMV35S:aroA/CP4*	NR	Cheng *et al.*, 2003
CPAN1676, PBW343	MSdC	LBA4404	pCAMBIA3301	*CaMV35S:gus, CaMV35S:bar*	6.7-8.7	Chugh and Khurana, 2003

Species/genotype	Explant	*Agrobacterium* strain	Binary vector	Promoter/gene	Transformation efficiency (%)	Reference
Bobwhite	PCIE	C58C1	pPTN115	*CaMV35S:gus, CaMV35S:nptII*	0.5-1.5	Haliloglu and Baenziger, 2003
Bobwhite	PCIE	C58(ABI)	pMON30120, pMON30174, pMON30139	*CaMV35S: aroA/CP4, ScBV: aroA/CP4, Act1:aroA/CP4*	4.4	Hu *et al.*, 2003
Veery5	IEdC	LBA4404	pHK21, pHK22		NR	Khanna and Daggared, 2003
Bobwhite, Canon, Florida, Cadenza	IE	AGL1	pAL156-pAL154	*Ubi1:gus, ubi1:bar*	0.3-3.3	Wu *et al.*, 2003
Bobwhite	PCIE	C58(ABI)	pPV-TXGT10	*Act1:aroA/CP4, CaMV35S:aroA/CP4*	NR	Zhou *et al.*, 2003
Vesna	IE	LBA4404, AGL1	pTOK233, pDM805	*Nos:nptII, CaMV35S:gus, CaMV35S:hpt, Act1:gus, ubi1:bar*	0.13 (LBA4404), 0.41 (AGL1)	Mitic *et al.*, 2004
Kontesa, Torka, Eta	IE	AGL1, EHA101, LBA4404	pDM805, pGAH, pTOK233	*CaMV35S:gus, Act1:gus, Ubi1:bar, Nos:nptII, CaMV35S:hpt*	1 (AGL1), 0.2-8.1 (EHA101), 0.2-2.3 (LBA4404)	Przetakiewicz *et al.*, 2004
Hesheng3, Yan103, Yanyou361	IEdC	GV3101	pROK2	*CaMV35S:nptII, CaMV35S:AtNHX1*	1.3-2.9	Xue *et al.*, 2004
Bobwhite, Sakha206, Yangmai10, PM97034	IE	C58(ABI), C58C1	pMP90, pPZP family	*CaMV35S:nptII, CaMV35S:gus*	0.18-3.5	Clemente and Mitra, 2005
Yangmai10	IEdC	C58C1	pCAMBIA3300	*Ubi1:Waxy*	NR	Li *et al.*, 2005
Shannong 9956049	IEdC	LBA4404	pROK2	*Nos:nptII*	1.18	Bi *et al.*, 2006
Keumkangmil, Alchanmil, Bobwhite	ME	KYRT1	pCAMBIA1305.1	*CaMV35S:hpt*	NR	Han *et al.*, 2006
HD2329, CPAN1676, PBW343	ME, MEdC	LBA4404	pBI101, pCAMBIA3303	*Act1:gus, CaMV35S:gus, Nos:nptII, CaMV35S:bar*	1.77	Patnaik *et al.*, 2006
Shiranekomugi	MSD	M-21, LBA4404	pIG121Hm, pBI-res, pBI-res2	*CaMV35S:gus, Nos:nptII, CaMV35S:hpt*	NR	Supartana *et al.*, 2006
Yan361, Yan2801, H11	MSD	EHA105	pBLG	*CaMV35S:nptII*	9.82	Zhao *et al.*, 2006
Yangmai158	PCIE		pCAMBIA3300	*CaMV35S:bar*	NR	Yu and Wei, 2008
Een1	MSD	LBA4404	NR	*CaMV35S:gus, CaMV35S:nptII*	3 to 31	Yang *et al.*, 2008

Species/genotype	Explant	*Agrobacterium* strain	Binary vector	Promoter/gene	Transformation efficiency (%)	Reference
Crocus, Chinese spring	Floral organs	C58C1, AGL1	pBECKSred	*CaMV35S:Lc/C1, Nos:nptII*	0.3-0.6	Agarwal *et al.*, 2009
Bobwhite	IE	NR	pMON42071, pMON42072, pMON66350	*Act1:aroA/CP4, Zm.hsp70:aroA/CP4*	2.8-5.7	Dan *et al.*, 2009
EM12	PCME	LBA4404	pBI121	*CaMV35S:gus, CaMV35S:nptII*	0.025	Ding *et al.*, 2009
Certo	IE	LBA4404	pSB187	*Ubi1:gfp, CaMV35S:hpt*	10	Hensel *et al.*, 2009
Yumai66, Lunxuan208, Bobwhite	ME, PCME	C58C1	pUbiGN	*Nos:nptII, Ubi:gus*	0.06 (Yumai 66), 0.67 (Lunxuan 208), 0.89 (Bobwhite)	Wang *et al.*, 2009
Crocus	SPK	C58C1, AGL1	pDS(Hys)35S, pBECKSred	*CaMV35S:Lc/C1, Nos:nptII, Nos:hpt*	0.44	Zale *et al.*, 2009
Gemmiza9, Gemmiza10	MEdC	LBA4404	pBI121	*CaMV35S:gus, CaMV35S:nptII*	6.9 (Gemmiza9), 8.7 (Gemmiza10)	Moghaieb *et al.*, 2010
Chakwal97, Inqilab91, Tartara2000	MEdC	EHA101	pTCL5	*CaMV35S:gus, CaMV35S:Xa21, CaMV35S:hpt*	NR	Raja *et al.*, 2010
Inqilab91	ME		pIG121Hm	*CaMV35S:gus, Nos:nptII, CaMV35S:hpt*	6.25-15.62	Rashid *et al.*, 2010
CPAN1676	Anther haploid embryos	LBA4404	pCAMBIA3301	*Act1: HVA1*	NR	Chauhan and Khurana, 2011
GA2002	apical meristem (*inplanta*)		pBI121	*CaMV35S:gus, Nos:nptII*	NR	Razzaq *et al.*, 2011
CB037, Kenong199, Xinchun9, Lunxuan987, Shi4185	IEdC	C58C1	pPTN290	*Ubi1:gus, CaMV35S:nptII*	0.058	Tao *et al.*, 2011
NB1	IMS	EHA105	pGBSSTP-EYFP	*Sc4:nptII, Act1:EYFP*	5.6 ± 1.2	Shaw and Gray, 2011
Kontesa, Torka	PCIE		pCAMBIA1305.2, pGreenII0000	*CaMV35S:gus, CaMV35S:nptII, CaMV35S:bar, Nos:nptII, Nos:bar*	3.58 (Kontesa), 3.14 (Torka)	Binka *et al.*, 2012
HD2329	MSD	GV2260	p35SGUSINT	*CaMV35S:gus, CaMV35S:nptII*	1.16	Chugh *et al.*, 2012

Species/genotype	Explant	*Agrobacterium* strain	Binary vector	Promoter/gene	Transformation efficiency (%)	Reference
Bobwhite	IEdC	C58C1	pAM4424	*CaMV35S:nptII, AMTP:BLF*	NR	Han *et al.*, 2012
ND146, JM6358	IEdC	LBA4404	pBCSL16	*CaMV35S:nia, CaMV35S:nptII*	1.68 (ND146), 0.40 (JM6358)	Zhao *et al.*, 2013
AARI-11, Aas-11, Dhurabi-11, Faisalabad-2008, Lasani-08, Millat-11, Pak-81, Punjab-11, Sahar-2006, Shafaq, V-07096, V-08203, VIII-83	MEdC	AGL1	pGA482	*gus, nptII*	NR	Abid *et al.*, 2014
T. dicoccum						
DDK1001, DDK1009	ME, IE	LBA4404	p35SGUSINT, pBI101	*CaMV35S:gus, Act1:gus, Nos:nptII*	NR	Khuranna *et al.*, 2002
DDK1001 (Emmer)	MSdC		pCAMBIA3301	*CaMV35S:gus, CaMV35S:bar*	6.9	Chugh and Khurana, 2003
T. durum						
Sohag2	SPK		pBI-P5CS	*CaMV35S:P5CS, CaMV35S:gus, Nos:nptII*	0.9	Sawahel and Hassan, 2002
PDW215, PDW233, WH896	ME, MEdC	LBA4404	pBI101	*Act1:gus, CaMV35S:gus, Nos:nptII, CaMV35S:bar*	1.28	Patnaik *et al.*, 2006
PDW215 (Pasta)	ME		pCAMBIA3301	*CaMV35S:gus, CaMV35S:bar*	3	Vishnudasan *et al.*, 2005
PDW215	MSD		pCAMBIA3301	*CaMV35S:bar*	0.84	Chugh *et al.*, 2012
T. turgidum						
Ofanto	IMS	AGL1	pAL154/pAL156	*Ubi1:gus, Ubi1:bar*	0.6-9.7	Wu *et al.*, 2008
Durum (Stewart)	IE		pAL156-pAL154	*Ubi:bar, Ubi:gus*	2.8-6.3	He *et al.*, 2010
Om Rabia3	PCME	GV3101	pCAMBIA1391Z	*PrDHN-5:gus, Ubi1:gus, Ubi1:bar*	NR	Amar *et al.*, 2013

Explants: *IM- immature embryos; ME- mature embryos; PCIE- pre-cultured immature embryos; PCME- pre-cultured mature embryos; IEdC- immature embryo-derived calli; MEdC- mature embryo-derived calli; SPK- spikelet; IMS- immature seedling; MSD- mature seedling; MSdC- mature seed derived calli; INFdC-inflorescence-derived calli; YE- young ears.*

Promoters: *Act1-Rice actin; AMTP-Chlorella virus (PBCV1) Adenine Methyltransferase; CaMV35S-Cauliflower mosaic virus; Nos-Nopaline synthase; PrDHN-5-A wheat dehydrin gene promoter, Sc4-subterranean clover stunt virus; ScBV-Sugarcane bacillirorm virus; Ubi1-Maize ubiquitin; Zm.hsp70-Zea mays heat-shock protein 70.*

Target/marker gene: *aroA/CP4-EPSPS (5-enolpyruwylshikimate-3-phosphate synthase); bar-Phosphinothricin acetyltransferase; BLF- Bovine lactoferrin; EYFP-Enhanced yellow fluorescent protein; gfp-Green fluorescent protein; gus- -glucuronidase; hpt-Hygromycin phosphotransferase; HVA1:is a group 3 LEA gene used in transformation for enhancing drought tolerance in both monocots and dicot plants; Lc/C1-Anthocyanin biosynthesis regulatory; nia:tobacco nitrate reductase; Nos-Nopaline synthase; nptII-Neomycin phosphotransferase II; P5CS-pyrroline-5-carboxylate synthetase; Waxy-GBSSI (granule bound starch synthase I); Xa21-bacterial blight resistance.*

NR-Not reported

5. Status of wheat transformation in India

In India, the wheat transgenic research is in very primal stage and has been initiated in early ninety's. Till now a few studies have been undertaken with respect to transformation of marker and gene of interest into wheat and are discussed in brief as below.

5.1 Transformation of marker gene: Mahalakshmi and Khurana (1995) used different explants of wheat and found that mature seeds were more responsive with agro strains A281 followed by A348 and GV2260 using *gus* gene activity in HD2329, Arjun and CPAN1676 genotypes of wheat. Chauhan *et al.,* (2007)showed a good regeneration system in 3 different species namely *T. Aestivum* (CPAN1676, HD2329 and PBW343), *T. durum* (PDW215, PDW233 and WH896) and *T. dicoccum* (DDK1001,DDK1025 and DDK1029) using thidiazuron in the media. A non-tissue culture method of seed transformation was demonstrated with HD2339 and PDW215 seeds of *T. aestivum* and *T. durum* with a transformation efficiency of 1.16% and 0.84% respectively using GUS marker with GV2260 and LBA4404 *Agrobacterium* strains (Chugh *et al.,* 2012). Recently, Habib (2014) developed a good *Agrobacterium* transformation system (upto 6%) with *gus* gene in wheat and showed that explants type, *Agrobacterium* strains and cell density, and T-DNA delivery are important in durum wheat transformation.

5.2 Transformation of herbicide resistance gene: Gopalakrishnan *et al.* (2000) has established highly efficient microprojectile bombardment method of wheat transformation in CPAN004, Sonalika and UP2338 varieties of Indian wheat and successfully transformed the *bar* gene to get putative transformants. Patnaik *et al.* (2006) developed the transformation of mature embryos of *T. aestivum* and *T. durum* with 200 µM acetosyringone and 2-3 days of co-cultivation with LBA4404 agro strain and putative transformants were obtained for *bar* gene (herbicide resistance) and proteinase inhibitor (*pin 2*) gene for insect resistance in wheat.

5.3 Transformation of abiotic stress resistance genes: Patnaik and Khurana (2003) showed particle bombardment method of gene transfer in *T. aestivum* (CPAN1676) and *T. Durum* (PDW215) with mature embryos. The transgenics with *HVA1* gene were developed and transformation was confirmed under controlled conditions in T_0 plants. Kasirajan *et al.,* (2014) transformed the *AtCBF3* gene into HDR77-A wheat variety with particle bombardment method and validated the putative T_1 plants under moisture stress conditions, which showed 8% yield advantage over wild type plants under stress.

6. Regulatory system and future of transgenic wheat

All new genetically modified (GM) crop varieties must go through a safety assessment process prior to commercialization. India established a strict regulatory system for the risk assessment of GM plants and worked alongside the Organization of Economic Cooperation of Development (OECD) and United Nations Environment Programme (UNEP) to make continuous improvements to this process. Guidelines for biosafety assessment are now largely imposed; GM crops in India have to pass through five phases of evaluation involving a proof of concept in the laboratory, the bio-evaluation in the green house, release to the confined environment and large-scale test in an open field and finally, authorization for field applications. Each step has its strict requirements following the same criteria as those of other countries. For example, before applying for environmental release the applicant needs to provide the data on the gene function, genetic and expression stability of the target gene and of the precise position of integration of transgenes in the wheat genome, evidence of a change in allergenicity or toxicity of the GM variety and potential for out-crossing pollination with related species. It is expected that this process normally takes at least 10 years for GM wheat to go from the laboratory to field production. However, there is a high potential to explore the area of chloroplast transformation, RNAi-based gene silencing and marker-free transformation system in wheat to meet future needs and demands.

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