Review Article

Genetic transformation of *Sorghum bicolor*

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ABSTRACT

Great millet (*Sorghum bicolor* (L.) Moench) is cultivated across the world for food and fodder. It is typically grown in semi-arid regions that are not suitable for cultivation of other major cereals. Sexual incompatibility and shortage of available genes in germplasm to combat biotic and abiotic stresses resulted in marginalized yields of this crop. Genetic modification of sorghum with agronomically useful genes can address this problem. Here, we tried to review and summarize the key aspects of sorghum transformation work being carried out so far by various research groups across the world. The approaches used and the obstacles in generating transgenic sorghum are also pointed out and discussed. **[Physiol. Mol. Biol. Plants 2009; 15(4) : 287-302]** *E-mail : vgirija_shank@yahoo.com; v.shankar@itc.in*

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INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth major cereal crop cultivated in 44.4 million hectares worldwide and accounts for 3.5 % of the total cereal grain production. It is cultivated in 71 countries across six continents with an estimated annual grain production of about 57.9 million metric tonnes (FAO Statistics, 2004). It is typically grown in areas with low soil moisture and high temperatures which are not suitable for growing other major cereal crops like rice, wheat and maize.

Sorghum is an important staple food crop, especially of the poor in Africa, Asia and Central America. It is the major source of fodder for animals and for ethanol production. Sorghum includes at least four groups of cultivated plants: grain sorghum; sweet sorghum for forage; Sudangrass for pasture, hay and silage; and broom corn for making brooms. The cultivated sorghum had its origin in Africa near the present-day Sudan and Ethiopia. It includes five basic races, viz., *bicolor, guinea, caudatum, kafir* and *dura* (Harlan and De Wet, 1972). Most of the global grain sorghum is produced in arid or semi-arid regions and is grown mostly as a rainfed crop. Unlike USA and Australia, marginal farmers in the tropics with low or no purchased inputs generally grow sorghum on small farms. Less input, poor crop management and several complex stress factors are responsible for the low yields of this crop. The sorghum cultivars have potential to produce more grain per unit area (Seetharama *et al.,* 2003) but their potential is not fully tapped.

In the recent years, conventional breeding for improved grain production resulted in the development of improved cultivars with significant increase in the productivity of this crop. However, several biotic and abiotic factors such as drought, insect pests, fungal pathogens and parasitic weed like *Striga* could not be combated efficiently through conventional breeding techniques alone. About 150 insect species and more than 100 plant pathogens are known to adversely affect sorghum grain production.

The shortage of useful genes available in the donor germplasm and the difficulty of making wide crosses because of sexual incompatibility marginalized the conventional breeding from making major impact on sorghum crop resistance to pests and diseases. The primary objective for most of the national and international sorghum improvement programs is to improve the yield, nutritional quality and to stabilize

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the sorghum production under dry land conditions. Agronomically, beneficial genes available across genera can be incorporated into the sorghum genome through genetic transformation technology along with *in vitro* techniques and generate new genotypes to meet the above requirement. Prerequisites for the application of *in vitro* techniques for crop improvement are efficient and reproducible methods of regeneration, stability and genotype independent protocols for genetic transformation. Though significant progress has been achieved, successful transfer of alien genes into cultivated sorghum have met with limited success.

Reports on transgenic sorghum obtained through four different gene transfer methods are known so far. They include: *Agrobacterium*-mediated indirect method followed by electroporation, particle bombardment and mild ultrasonication pollen-mediated transformation. Strength of promoters in driving the expression of various genes of interest was also reported (Able *et al.,* 2001; Hill-Ambroz and Weeks, 2001; Jeoung *et al.,* 2002; Tadesse *et al.,* 2003). Emani *et al.,* (2002) indicated that among the cereals, sorghum has been the most recalcitrant crop species to genetic modification and therefore, there are few reports available on sorghum transformation among which the majority have reported transgene silencing. Methylation-based transgene silencing and reactivation in sorghum is well documented. It can be said that much of the work by earlier investigators is mainly focused on the optimization of parameters for tissue culture and gene transfer methods, accessing the strength of various promoters, identification of efficient selection and reporter genes. Few attempts were made with agronomically useful genes, so far. The summary of reports on genetic transformation of sorghum have been presented in Table 1.

Strategies for successful transformation

Besides an amenable tissue culture and regeneration systems, the following factors are also critical for development of transgenic plants: (i) suitable target gene in a convenient vector with reporter (to conform alien gene introduction into target cell or tissue) and selectable (to eliminate those cells into which foreign DNA has not been incorporated) marker genes; (ii) method of DNA delivery into the cell (iii) efficient testing method to confirm transformation events (stable integration) and (iv) adopting strategies to address transgene silencing. It is equally important to ensure consistent inheritance and expression of the transgene in the progeny. Lack of pleotropic effects and consideration of biosafety issues are important before useful transgenic can be commercialized.

Explants for transformation and plant regeneration

Protoplast, suspension cell cultures, immature embryos, immature inflorescences and shoots tips from germinating seedlings are used as explant material to introduce various transgenes into sorghum genome. The first report of direct DNA uptake into protoplasts of sorghum by electroporation was carried out by Ou-Lee *et al.,* (1986) and latter by Battraw and Hall (1991). Hagio *et al.,* (1991) reported stable transformation from suspension cell cultures of *Sorghum vulgare* through microprojectile bombardment. Though these above investigators reported expression of the integrated foreign gene in detectable amounts, attempts to regenerate them in to whole plants were not attempted. Bombardment of cell suspension cultures directly has advantage as it eliminates the need for preparing protoplasts and reduces the formation of chimeras which are often seen when embryos are bombarded. The disadvantage of the protoplast or cell suspensions is that the method is laborious, needs special skills, tends to be cultivar specific and have very low regeneration frequency.

Casas *et al.* (1993) were first to report successful transformation of sorghum immature embryos via biolistic method of gene transfer. They reported that among the surviving calli, only a few exhibited shoot development indicating regeneration of plants at low frequency. Since then, immature embryos or immature embryo derived calli has been the more popular explant for sorghum transformation (Zhu *et al.,* 1998; Rathus and Godwin, 2000; Zhao *et al.,* 2000; Hill-Ambroz and Weeks, 2001; Jeoung *et al.,* 2004). The frequency of plant regeneration reported so far, does not seem to be sufficient for genetic transformation on a routine basis (Harshavardhan *et al.,* 2002).

Shoot tips from germinating seedlings are also widely used explants in sorghum transformation (Tadesse and Jacobs, 2004; Tadesse *et al.,* 2003; Gray *et al.,* 2004; Devi *et al.,* 2004). Explants derived from meristematic tissues at the early stages of development are most amenable to tissue culture conditions. Tissues such as seed embryos and shoot apices isolated from germinated seedlings are readily accessible and form year long source for cereal explants. Highly uniform meristematic tissues are desirable for genetic transformation to minimize chimeras and somaclonal variants. So, meristematic shoot tip along with a pair of primordial

Table 1. Summary of attempts on genetic transformation of sorghum so far.

S. No.	Transformation method	Explant/ culture system	Trans-gene	Promoter	Selection agent	Report	Reference
1	Electroporation	Protoplasts	*cat*	CaMV 35S/ *copia* promoter of drosophila	Chloramphe-nicol	Efficient gene expression under both promoters	Ou-Lee *et al.,* 1986
2	Electroporation	Cell suspension/ Protoplasts	*npt II*	CaMV 35S	Kanamycin, 100 mg L^{-1}	Stable trans-formation	Battraw and Hall, 1991
3	PDS-1000/He (Bio-Rad)	Cell suspension culture	*npt II, hpt, uid*A	*adh 1*/CaMV 35S	Kanamycin/ hygromycin	Stable trans-formation	Hagio *et al.,* 1991
4	PDS- 1000/He (Bio-Rad)	Immature embryos	*bar, uid*A	CaMV 35S	Bialophos, 3 mg L^{-1}	Plants regen-erated at low frequency	Casas *et al.,* 1993
5	PDS- 1000/He	Immature embryos/ inflorescence callus	*bar, uid*A *& luc*	–	Bialophos	Plants regen-erated at low frequency	Kononowicz *et al.,* 1995
6	PIG (Particle-Inflow Gun)	immature embryos/ Inflorescence derived callus	*bar*	CaMV 35S/ *act 1*	Bialophos/ 2 mg L^{-1}	Single plant reported	Rathus *et al.,* 1996
7	PDS-1000/He	Immature inflorescence	*bar, uid*A	CaMV 35S	Bialophos	Plants regen-erated at low frequency	Casas *et al.,* 1997
8	PDS- 1000/He	Immature embryos	*bar/ chitinase1*	CaMV 35S	*Basta*/1-2 mg L^{-1}	Showed SB positives	Zhu *et al.,* 1998
9	PIG	Immature embryo	*bar*	CaMV 35S/ *act 1*	*Basta*/1-2 mg L^{-1}	Casein hydroly-sate used for increasing regeneration frequency	Rathus and Godwin, 2000
10	*Agrobacterium* (LBA4404)	Immature embryos callus	*bar*	*ubi1*	PPT/5 mg L^{-1}	2.1% trans-formation frequency reported	Zhao *et al.,* 2000
11	Particle bombardment	Immature embryos callus	*uid*A, *bar, gfp*	*act1, ubi1,* CaMV35S	Bialophos/2 mg L^{-1}	*ubi>act1>* CaMV35S	Able *et al.,* 2001
12	Particle bombardment	Immature embryos calli	*uid*A	*ubi1, act1, adh1,* CaMV35S*:* CaMV35S	Nil	*CaMV35S> ubi1>act1> adh1*	Hill-Ambroz and Week, 2001

Table 1. Continued .....

S. No.	Transformation method	Explant/ culture system	Trans-gene	Promoter	Selection agent	Report	Reference
13	Particle bombardment	Immature embryos callus	*uid*A, *bar*	*act1, ubi1*	PPT/5 mg L^{-1}	Methylation based transgene silencing	Emani *et al.,* 2002
14	Particle bombardment/ *Agrobacterium*	Immature embryos and leaves	*uid*A, *gfp*	*ubi1, act1, adh1,* CaMV35S	Observing GFP expression	*ubi>CaMV35S >Act1>adh1*	Jeoung *et al.,* 2002
15	Particle bombardment	Immature embryos and shoot tips	*uid*A, *bar, neo*	*ubi, act1, adh1,* CaMV35S	Geneticin/75 mg L^{-1} and 100 mg L^{-1}	*ubi>act1>adh1 >CaMV35S*	Tadesse *et al.,* 2003
16	PDS-1000/He	Shoot meristems	*bar/ HVA I*	CaMV35S	Glufosinate/ 10 mg L^{-1}	For drought tolerance. SB conformed.	Devi *et al.,* 2004
17	PIG	Shoot meristems	*bar/ cry1Ab & cry1B*	CaMV35S/ *act 1*	*Basta*/2 mg L^{-1}	For insect resistance. SB not reported	Gray *et al.,* 2004
18	PDS-1000/He	Immature embryos/ Shoot tips	*npt II, dhdps-raec 1*	*act 1/adh1/* CaMV 35S, *ubi1*	Kanamycin/ For more lysine content	Obtained 13 plants. Showed Southern positive	Tadesse and Jacobs, 2004
19	*Agrobacterium*-mediated	Immature embryo callus	*hpt, npt, uid*A	35S	Hygromycin	SB reported	Carvalho *et al*., 2004
20	*Agrobacterium* (LBA4404)	Immature embryo callus	*gfp/bar/ t1p, rice chitinase G11*	*ubi1*	Bialophos/3 mg L^{-1}	SB reported	Jeoung *et al*., 2004
21	Particle bombardment	Shoot tips	*uid*A, *bar, cry1Ac*	*mpiC1*	Basta 2 mg/l	PCR, SB and ELISA	Girija-shankar *et al.,* 2005
22	Particle bombardment	Shoot tips	*uid*A/*bar/ cry1Ab/ cry1Ac*	*ubi1*	Basta 2 mg/l	PCR, SB and ELISA	Girija-shankar, 2005
23	*Agrobacterium*-mediated	Immature embryo callus	*gfp*/*tlp*	*ubi1*	No marker	SB for *tlp* gene reported	Gao *et al.,* 2005
24	*Agrobacterium*-mediated	Immature embryo callus	*gfp, man*A	*ubi1*	Mannose sugar added in medium	SB and Western blots for *man*A	Gao *et al.,* 2005

Table 1. Continued .....

S. No.	Transformation method	Explant/ culture system	Trans-gene	Promoter	Selection agent	Report	Reference
25	*Agrobacterium*-mediated	Immature embryo callus	*npt II, uid*A	–	Geneticin or Paromomycin	–	Howe *et al.,* 2006
26	*Agrobacterium*-mediated	Immature embryo callus	*hpt*	–	Hygromycin	SB reported	Nguyen *et al*., 2007
27	Mild ultra-sonication	Pollen	*npt II, uid*A	–	–	PCR and SB reported	Wang *et al.,* 2007
28	*Agrobacterium*-mediated	Immature embryo callus	*gfp, man*A	*ubi1*	Mannose	PCR, Western and SB reported	Gurel *et al.,* 2009
29	*Agrobacterium*-mediated co-transformation	Immature embryo callus	*bar*, sorghum lysyl tRNA synthetase	CAMV 35S, maize zein CZ19 B1	Phosphino-thrithin, PPT	PCR and SB reported	Lu *et al*., 2009

Abbreviations: cat- Chloramphenicol acetyl transferase, *npt II*- Neomycin phosphotransferase; *bar*- Bailophos resistance; *gfp*-green fluorescence protein; *hpt*- Hygromycin phosphotransferase; *act* 1- Rice *actin* promoter; *ubi* – Maize *ubiquitin1* promoter; *adh* 1- alcohol dehydrogenase promoter; CaMV35S – Cauliflower mosaic virus 35S promoter; PPT- Phosphinothricin; SB-Southern Blots.

leaves are reprogrammed *in vitro* to produce multiple shoots or large number of somatic embryos. Eventually, high regeneration frequency and efficient protocols for *in vitro* regeneration of sorghum have been developed which formed the basis for genetic transformation studies (Harshavardhan *et al.,* 2002; Girijashankar *et al.,* 2005).

Bombarding of immature inflorescence derived callus is followed but the regeneration frequency being low, made it least preferred explant (Kononowicz *et al.,* 1995; Rathus *et al.,* 1996; Casas *et al.,* 1997). Of late, the simple and easy way of generating transgenic sorghum with the use of transformed pollen is developed (Wang *et al.,* 2007). In this method, pollen from sorghum was transformed by a novel genetic transformation approach using mild ultrasonication. The treated pollen is then used to pollinate stigmas of desired genotypes. Taking into consideration the different explants used, cultured immature embryos and shoot tips of sorghum are the two explants of choice which have been predominantly used for sorghum genetic transformation.

Role of promoters and markers in transformation

Strength of promoters in transgene expression

Efficient transgene expression requires presence of a suitable promoter and terminator. Heterologous promoters such as CaMV35S, rice *actin1*, maize alcohol dehydrogenase - *adh1, copia* long terminal repeat promoter of Drosophila, maize polyubiquitin 1-*ubiquitin1* have been shown to regulate the expression of transgenes in sorghum. The *actin1* gene promoter from rice and *ubiquitin1* gene promoter from maize are the two monocot promoters that showed high constitutive activity in sorghum.

Depending upon the plant species being examined, the promoter will also be an important factor in evaluating optimal transgene expression (Able *et al.,* 2001). In general, the CaMV35S promoter is strong in dicot species while its expression level is much lower in monocotyledons. Research on different promoters linked to the *uid*A and/or *gfp* reporter genes has been carried out to identify the most suitable promoter for sorghum.

It was hypothesized that poor promoters may be one of the potential reasons for lack of successful transgenic sorghum plants. Optimal promoter sequences should be identified to increase the transformation frequency in sorghum. A more effective promoter would dramatically increase the number of transformation events recoverable among sorghum cells (Hill-Ambroz and Weeks, 2001). During the early days of sorghum transformation studies, CaMV35S and maize a*dh1* promoters were widely used. From 2000 onwards, maize *polyubiquitin1* gene promoter *ubi1* evolved to be the prominent controlling element which enhanced the transgene expression in sorghum. The dual 35S of cauliflower mosaic virus, rice *actin-1*, maize alcohol dehydrogenase-1 (*adh1*) and maize *ubiquitin-1* promoters driving the GUS gene expression was compared by Hill-Ambroz and Weeks (2001). They reported that the level of quantified GUS expression was much higher in wheat embryos than in sorghum embryos when similar gene constructs are used and concluded that none of the promoters could generate sufficient expression to allow sorghum transformation to occur at a practical frequency.

During the same year, Able *et al.,* (2001) investigated the transient GUS expression under the control of these promoters (*ubiquitin1*, *actin1* and CaMV35S) and reported that a significantly higher number of GUS spots were obtained with *ubiquitin1* promoter when compared to *actin1* and CaMV35S promoters. Similar studies were also carried out by Tadesse *et al.,* (2003) in sorghum with immature embryos and shoot tips using four different promoters (*ubiquitin1*, *actin1*, *adh1* and CaMV35S) for transient GUS expression. They performed both GUS histo-chemical staining and enzyme activity assay. According to them, the strength of these promoters in sorghum was determined to be in the order: *ubi1*>*act1D*>*adh1*>CaMV35S.

Jeoung *et al.,* (2002) in their attempt to optimize the parameters for use of *gfp* and *uid*A as visual markers in sorghum transformation, evaluated different promoters controlling the expression of these two marker genes. The order of the promoter strength as measured by green fluorescent protein (GFP) expression in calli and was in the order of *ubi1*>CaMV 35S>*HBT* (HBT-a chimeric promoter with the 35S enhancer fragment fused to the basal promoter that includes the TATA box, transcription initiation site and 5' untranslated region). On the other hand, the order of promoter strength for GUS expression was *ubi1*>CaMV 35S>*act1*>*adh1*. The list of various promoters used for transformation studies in sorghum are given in Table 1. The *ubiquitin1* and *actin1* promoter constructs have one native intron incorporated in the transcription unit, which has been implicated in elevating the mRNA abundance and enhancing the gene expression in transformed cereal cells.

Wound-inducible promoter

The use of wound-inducible promoters to direct the expression of genes encoding insecticidal proteins in transgenic crops has been proposed but rarely documented in monocots. These promoters can save energy of the plant and delay the occurrence of resistance among the target insect population (de Maagd *et al.,* 1999).

Like other wound-inducible proteinase inhibitors, whose expression is also developmentally regulated in storage and/or reproductive organs, MPI protein accumulates in maize embryos. Such features make it an attractive candidate to direct the expression of candidate genes in transgenic plants apart from the normal wounding response in any tissue of the plant. Breitler *et al.* (2001) reported the wound-inducible expression of a cry1B crystal protein (0.1-0.2 % of total soluble protein) under the control of maize proteinase inhibitor (*mpi*) promoter extending from –689 to +197 and named as C1 fragment which afforded full protection to transgenic rice plants and showed 100 % mortality of stripped stem borer second instar larvae.

Using the same promoter, Girijashankar *et al.,* (2005) genetically transformed sorghum (genotype BTx 623) using particle bombardment method. Insect bioassays (leaf disc assay) of T_1 generation plants carrying *mpi-cry1Ac* showed reduction in leaf damage (60 %), larvae weight (25 %) and increased larval mortality (40 %) when compared to the control plants. In response to mechanical wounding, the Cry1Ac toxin is expressed at low levels in leaves (1-8 ng/g of leaf tissue). This is the first report on study of a transgene under wound-inducible promoter in sorghum. Also, it is the first report on transgenic sorghum expressing synthetic Bt toxin.

On the other hand, *cry* genes under the control of the constitutive promoter from maize i.e., maize *polyubiquitin1* promoter could drive the expression at low levels compared to the wound-inducible promoter *mpiC1*. From the preliminary studies, the wound-inducible maize protease inhibitor promoter (*mpiC1*) was found to be 14.55 fold stronger in expressing the transgene *cry1Ac* than the *ubiquitin1* promoter (Girijashankar, 2005). As the number of transgenic events compared is very low, this work is incomplete and needs further study. Under the control of the same

constitutive promoter (*ubi1*), the composition of the transgene showed significant difference in protein expression levels. The *Bt* gene *cry1Ab* showed 3.64-fold increase in the δ-endotoxin production compared to *cry1Ac* in the young leaves of T_0 plants, while these two genes are 95 % similar in the nucleotide sequence. A difference of 5 % in the nucleotide sequence resulted in a noticeable difference (>3 fold) in the transgene expression levels.

Reporter genes in sorghum transformation:

Many reporter systems are available for detecting a successful event of genetic transformation. Among them, so far four reporter systems were used in different sorghum transformation studies so far. They include, *uid*A gene coding for β-glucuronidase (GUS), the anthocyanin pigmentation system R and C1 of maize, the fire-fly luciferase *luc* gene system and the green-fluorescent protein (GFP) *gfp* system. GUS is the most widely used reporter system in sorghum transformation starting from initial transformation attempts (Hagio *et al*., 1991) till the latest reports of Wang *et al.,* 2007). The main disadvantage of using GUS as a reporter system is that it requires a destructive assay that precludes further proliferation and regeneration of identified transformed tissues.

The second most important reporter system is GFP which acquired importance because of its non-destructive visualization systems that can facilitate the recovery of identified transformed tissues (Gurel *et al.,* 2009; Gao *et al.,* 2005a, b; Jeoung *et al.,* 2004; Able *et al.,* 2001). Green fluorescent protein, comprises of 238 amino acids (26.9 kDa), originally isolated from the jellyfish *Aequorea victoria*. The use of *gfp* as reporter system in transformation studies along with agronomically important genes proved to be a failure in sorghum as no somatic embryos were formed on the sectors selected on bialophos co-bombarded with *gfp* and *bar* genes. Further investigations are needed to ascertain the toxicity of GFP to sorghum cells (Able *et al.,* 2001; Jeoung *et al.,* 2002). The other disadvantage in using GFP is the need for costly equipment like fluorescent stereomicroscope for detection of GFP. However, both the reports suggested that GFP was superior over GUS and can be used for early and reliable detection of transgenic events in efficient transformation protocols.

In contrast, the main advantage of using *uid*A gene as reporter system is the non-involvement of expensive equipment and ease of detection that involves visualization of the GUS expression (*uid*A) after treatment with the substrate, X-Gluc. However, use of R and C1 maize anthocyanin regulatory elements (Casas *et al.,* 1993) and *luc,* a fire fly luciferase (Kononowicz *et al.,* 1995) as reporter genes in sorghum transformation is also reported. Apart from these individual reports, these genes were not used in sorghum transformation studies.

Role of selectable markers

The development of reliable transformation system for the production of transgenic sorghum plants depends on the efficient expression of the introduced selectable marker genes (Tadesse *et al.,* 2003). The key to establish a successful transformation strategy lies in the adoption of an effective and foolproof selection strategy. The usefulness of a selectable marker gene to optimize sorghum transformation system that will eventually allow the introduction of agronomically important traits to sorghum by genetic transformation was demonstrated (Casas *et al.,* 1993; Harshavardhan *et al.,* 2003).

Transgenic sorghum tissues growing in vitro are screened against three broad categories of selection markers such as antibiotics, herbicide and nutrient assimilation. Five different selection markers were utilized in sorghum transformation. They include *cat, npt II, hpt, bar* and *man*A. Neomycin phosphotransferase II (*npt II*) gene isolated from *E.coli* conferring resistance to the antibiotic Kanamycin is one of the commonly used selection strategy for sorghum (Howe *et al.,* 2006; Tadesse and Jacobs, 2004; Battraw and Hall, 1991). Hagio *et al.,* (1991) reported the use of Hygromycin B phosphotransferase (*hpt*) as well as Neomycin phosphotransferase II (*nptII*) genes to confer Hygromycin and Kanamycin resistance, respectively.

Till date, the most successful and popular selection marker is the *bar* gene, derived from *Streptomyces hygroscopicus* which encodes the enzyme phosphinothricin acetyltransferase (PAT) conferring resistance to the herbicide phosphinothricin (PPT) or its analogues *Basta* (with its active ingredient glufosinate ammonia) or bialophos (Harshavardhan *et al.,* 2003).

The selectable marker *bar* gene is isolated from *Streptomyces hygroscopicus* codes for phosphinothricin acetyl transferase (PAT) proteins of 183 amino acids and shows 85 % DNA sequence homology with another marker gene *pat* isolated from *S. viridochromogenes*. Phosphinothricin inhibits glutamine synthetase (GS) irreversibly, resulting in inhibition of amino acid biosynthesis. Almost all the transformation studies

conducted earlier in sorghum used *bar* gene as selectable marker gene except for Battraw and Hall (1991) who used *nptII* and Hagio *et al.* (1991) where *nptII* and *hph* genes were used. The herbicide phosphinothricin is used as selection agent at a concentration of 5.0 mg L^{-1} (Zhao *et al.,* 2000) while others used low concentrations of the same i.e. 2-3 mg L^{-1} (Rathus *et al.,* 1996; Casas *et al.,* 1993). Bialophos (a tripeptide) and Basta (shows nonsystemic and localized effect) are the derivatives of PPT. Upto 3.0 mg L^{-1} of Bialophos (Jeoung *et al.,* 1999) and 2.0 mg L^{-1} Basta (Zhu *et al.,* 1998; Gray *et al.,* 2004) was used in the culture medium for selection of putative transformants. Cassas *et al.,* (1997) reported that in the absence of bialaphos in the selection medium the morphogenesis of sorghum immature inflorescences was primarily via embryogenesis while on the other hand, organogenesis was more predominant in callus maintained on herbicide selection. So, the selection agent used can influence the transgenic plant regeneration pathway in sorghum.

Recently, *man*A gene from *Escherichia coli* coding for phosphomannose isomerase enzyme, is used as the selectable marker gene while the disaccharide mannose is the selection agent (Gurel *et al.,* 2009; Gao *et al.,* 2005 b). The conversion of mannose to a metabolizable fructose carbon source is beneficial to plants. This is an efficient and non-destructive method of screening the transformed sorghum plants under *in vitro* conditions and is gaining popularity.

Methods of sorghum genetic transformation

Tissue culture is an enabling technology from which many novel tools have been developed to assist plant breeders. These tools can be used to increase the speed or efficiency of the breeding process, to improve the accessibility of existing germplasm and to create new variation for crop improvement (Able *et al.,* 2001). Genetic modification in plants is dependent on efficient *in vitro* regeneration protocols for development of transgenic events.

Plant transformation is performed using a wide range of techniques such as *Agrobacterium* Ti plasmid vectors, microprojectile bombardment, electroporation, microinjection, chemical (PEG) treatment of protoplasts. Though all methods have advantages that are unique to each of them, transformation using *Agrobacterium* and microprojectile bombardment are currently the most extensively used approaches. Owing to the difficulty in *Agrobacterium* mediated gene transfer, biolistic approach has been used extensively for the genetic transformation of the monocot species (Christou, 1995). However, for production of transgenic plants with single or low copy inserts, *Agrobacterium*-mediated transformation is most preferred among the research groups.

Electroporation

The first attempt to transforming sorghum was done by using electroporation method of gene transfer into sorghum protoplasts (Ou-Lee *et al.,* 1986), where transient expression of *cat* gene that coded for chloramphenicol acetyl transferase (*cat*) was reported. Battraw and Hall (1991) electroporated protoplasts isolated from embryogenic suspension cultures to introduce *nptII* along with *uid*A reporter gene. They studied, transient reporter genes expression using different factors such as linearization of the plasmid and the effect of electroporating with two different gene constructs. They obtained about 77 different Kanamycin resistant calli. However, plant regeneration could not be achieved. This method is out-dated with the development of superior and less cumbersome transformation methods like particle bombardment and *Agrobacterium*-mediated transformation.

Agrobacterium-mediated transformation

Agrobacterium–based DNA transfer system offers many unique advantages in plant transformation: (1) the simplicity of *Agrobacterium* gene transfer makes it a poor man's vector. (2) A precise transfer and integration of DNA sequences with defined ends. (3) linked transfer of genes of interest along with the transformation marker. (4) The higher frequency of stable transformation with single copy insertions. (5) Reasonably low incidence of transgene silencing. (6) The ability to transfer long stretches of T-DNA.

Genetic transformation of crop species using *Agrobacterium* is believed to be more practical, as the success rates of transformation are greater than biolistic approach. Further, unlike later, complex equipment is not involved. However, for a long period of time monocotyledons have been considered outside the host range of *Agrobacterium*. But, advances in understanding the biology of the infection process and the availability of suitable gene promoters as well as selectable markers improved transformation in monocotyledons. Initially, Godwin and Chicwamba (1994) reported inoculation of sorghum meristem tissue with *Agrobacterium.* Starting from this work, there are more than eight available reports on sorghum transformation with *Agrobacterium*.

Zhao *et al.* (2000) for the first time successfully carried out genetic transformation of sorghum through *Agrobacterium*-mediated *bar* gene delivery and obtained overall transformation frequency of 2.1 %.

Following the same method, Visarada *et al.* (2003) attempted genetic transformation of sorghum and reported transient GUS expressions. Their studies using *Agrobacterium*-mediated transformation showed that immature embryos and calli derived from immature inflorescences were ideal target tissues for obtaining high GUS expression. The latest work by Lu *et al.,* (2009) deals with the development of marker free transgenic sorghum plants harboring lysyl tRNA synthetase gene for enhanced lysine content in sorghum seed.

Biolistic or Particle or Microprojectile bombardment method

Of the various other methods for DNA delivery, microprojectile bombardment is the most widely deployed method for genotype-independent sorghum transformation. This method was first used to deliver the DNA and RNA into epidermal cells of *Allium cepa*. Particle bombardment is an efficient method of genetic transformation of cereals; where in biological molecules are driven at high velocity into the target tissue. It offers advantages such as introduction of multiple genes, simplicity of introducing transgenes and transformation in those plants where agro-infection is difficult. In the 90's, Sanford reported this process from Cornell University (Sanford *et al.,* 1993).

Initially, Hagio *et al.* (1991) reported transient expression of reporter genes in sorghum suspension cells by particle bombardment. During the first decade of sorghum transformation studies, there are only a few reports of successful recovery and analysis of transgenic sorghum plants (Casas *et al.,* 1993; Casas *et al.,* 1997; Zhu *et al.,* 1998). So far, PDS-1000/He gene delivery device of *Biorad* laboratories, Richmond, California has been the most common and successful device used for direct gene transfer. However, the device is expensive and has high operational costs. Further, there are complications related to intellectual property rights, as the device is only licensed and not sold. As alternative, different laboratories started using, a particle-inflow gun (PIG) constructed indigenously with the help of scientists of University of Queensland in Australia, following the design of Finer *et al.* (1992).

Optimal bombardment parameters

To optimize DNA delivery and minimize tissue damage, several parameters must be evaluated that include the microprojectile size, attachment of the DNA to the microprojectiles, distance to the target tissue and velocity of gas flow, including the effect of pressure, aperture and pulse time (Able *et al.,* 2001). Production of transgenic plants by particle bombardment can be divided into two processes: (i) introduction of DNA into cells with minimum tissue damage and (ii) Regeneration from transformed cells. Transformation is also affected by bombardment pressure, flight distance, amount of particles, DNA used per shot, the number of shots per target, donor plant variables like temperature, photoperiod, humidity, nature of explants etc. (McCabe and Christou, 1993).

Optimization of physical and biological parameters can increase the efficiency of these processes (Birch and Bower, 1994). Tungsten particles are less expensive but are more heterogeneous in size compared to those of gold. However, the major disadvantage of using tungsten is that it can catalytically degrade DNA over a period of time and may be toxic to some cell types. The optimal parameters for the indigenously built particle in-flow gun used in the recent studies were found to be 12 kg $cm_{É}^{2}$ of helium gas pressure and a flight distance of 7 cm between the target explant and the DNA holder for stable and efficient gene delivery (Harshavardhan *et al.,* 2002, Girijashankar *et al.,* 2005).

Mild ultrasonication pollen-mediated transformation

This latest method was reported by Wang *et al.,* (2007) from China. Pollen from sorghum was transformed by mild ultrasonication. Plasmid (*npt*II and *uid*A) and pollen from sorghum (genotype A(2)V4B) were submerged in a 0.3 mol/l sucrose solution and then subjected to ultrasonication. The treated pollen were then used to pollinate the stigmas of the male sterile line A(2)V4A. Transient gene expression studies and molecular analysis revealed that both of the transgenes are stably integrated into sorghum genome. These results indicate that direct gene transfer to pollen can be mediated by mild ultrasonication. The basic advantage of this method lies in eliminating the need of a tedious *in vitro* regeneration protocol in place. It is simple and requires less time but has the disadvantage of not having control on the number of transgene copies that get integrated into the sorghum genome.

Sorghum transformation studies till date

Initial steps in transformation

Improvement in field of sorghum transformation has been hampered by difficulties associated with tissue culture, recalcitrance to genetic transformation, low regeneration frequencies, non-availability of efficient protocols for transformation, transgene silencing and chimerism (Jeoung *et al.,* 2002, Girijashankar 2005, Girijashankar *et al.,* 2007). The first attempt of successful transgene integration into sorghum is by electroporating its protoplasts by Ou-Lee *et al.,* (1986) and latter by Battraw and Hall, (1991). In the same year the particle bombardment of a non-regenerable cell suspension was reported (Hagio *et al.,* 1991). Later over a period of two decade, different research groups have reported the production of transgenic sorghum plants for tolerance to pests, diseases, herbicides and to drought by using direct and indirect gene transfer methods.

Herbicide resistance

Only one herbicide resistant gene *bar* was used in the history of great millet transformation study, so far. The first transgenic sorghum plants are obtained by microprojectile bombardment of immature embryos with *bar* gene (Casas *et al.,* 1993). Immature inflorescence-derived calli was also transformed with similar procedure (Casas *et al.,* 1997) and more than fourteen reports are available regarding transformation of sorghum with *bar* gene. It is reported that bialophos resistance with *bar* gene was associated with low plant regeneration frequency under *in vitro* conditions where biolistic method of gene transfer is followed to introduce this gene into immature embryos and immature inflorescence derived calli (Kononowicz *et al.,* 1995). Finally, with constant efforts, a single plant was generated (Rathus *et al.*, 1996).

Nutritional quality improvement

Sorghum grain is loaded with starch and is relatively poor in protein and lipid. Tadesse and Jacobs (2004) with an aim to improve the amino acid content (lysine) of sorghum grain tried to modify the regulation of lysine branch of aspartate metabolic pathway. The de-regulation process involves the introduction of a mutated *dhdps-rl* gene encoding a feedback-insensitive dihydro-picolinate synthetase enzyme leading to accumulation of more amount of lysine. They obtained less than ten plants for the nutritional quality improvement through microprojectile bombardment of immature embryos and shoot tips with *dhdps-r1* gene. However, towards maturity, the lysine content in the transgenic lines was almost comparable with that of the control. Efforts are also underway to transfer the high molecular weight (HMW) wheat glutenin gene *1Ax1* into sorghum to alter dough quality for baking industry.

Recently, work on *Agrobacterium*-mediated co-transformation and regeneration from immature embryo callus, Lu *et al*., (2009) reported transgenic recovery of sorghum plants harboring a modified tRNAlys (from *Arabidopsis thaliana*) and sorghum lys1 tRNA synthase elements (TC2 or SKRS) for improving lysine content in sorghum seeds. The SKRS fragment is under the control of CZ19 B1 element of maize 19KDa zein protein. Though they could successfully generate transgenic sorghum plants, the expression of the lysine gene or amino acid content in sorghum seed is not reported.

Nutrient selection marker

Sorghum cultures cannot use mannose as a sole carbohydrate source, but can utilize fructose for that purpose. Phosphomannose isomerase (PMI) can convert mannose to fructose. The PMI structural gene (*man*A) of *Escherichia coli*, which is capable of converting mannose to fructose, has been used as a selectable marker gene. The conversion of mannose to a metabolizable fructose carbon source which is beneficial to plants was achieved in sorghum with *Agrobacterium*-mediated system using *man*A gene (Gao *et al.,* 2005a; Gurel *et al.,* 2009).

Drought tolerance

To impart resistance to abiotic stresses like drought, *HVA1* gene from barley was inserted into sorghum genome through biolistic transformation. Barley group 3 LEA protein i.e *HVA1* gene products gets accumulated under stress induced by ABA, dehydration, salt and extreme temperatures. This gene is related to drought tolerance and was introduced into shoot tip explants of germinating seedlings of sorghum cultivar RW 5023 via biolistic method and transgenic plants were successfully (Devi *et al.,* 2004). However, bioassays on drought tolerance is not reported.

Ectopic expression of *mtl*D gene which leads to the biosynthesis of mannitol can improve the tolerance to water stress and salinity. At Directorate of Sorghum Research (DSR), Hyderabad, India, a group of researchers are attempting to develop transgenic sorghum for improved salinity tolerance using *mtl*D gene (Balakrishna *et al*., 2007).

Disease resistance

Biolistic method of direct gene transfer is used to introduction rice *chitinase* and *bar* gene into sorghum chromatin and transgenic plants conferring resistance to fungus and herbicide (Basta) spray were generated (Zhu *et al.,* 1998). Stable expression of *chitinase* gene was detected in T_2, T_3 and T_4 progenies. Due to the inherent constraints associated with biolistic method, this group further standardized *Agrobacterium*-mediated protocol to transfer PR (pathogenesis related) genes into sorghum genotypes. Following this method, they could introduce two PR genes namely, rice *chitinase* (*G11*) and *tlp* (Thaumatin-like protein) into three different inbred lines of sorghum (Jeoung *et al.,* 2004). The transgenic plants showed moderate levels of resistance towards fusarium stalk rot disease. This research group from Kansas State University later attempted to enhance resistance to fungal diseases and drought tolerance through *tlp* gene from rice (Thaumatin-like protein). Along with the visual reporter gene *gfp,* the *tlp* was introduced into explants (immature zygotic embryos) of three sorghum genotypes (two inbreds: Tx 430 and C401; and a commercial hybrid, Pioneer 8505) via *Agrobacterium*-mediated transformation method (Gao *et al.,* 2005b) and eventually generated transgenic sorghum plants.

Pest tolerance and *cry* genes

About 150 insect species and more than 100 plant pathogens have been reported affecting sorghum. Sorghum producers face a major threat from insect pests and the annual estimated grain loss from pests is around US $1 billion (Nwanze *et al.,* 1995). To address the problem of pests, the first step towards insect resistance in sorghum was attempted by Gray *et al.,* (2004) where they tried to introduce *cry1Ab* and *cry1B* genes into the sorghum genotype P898012. However, they did not confirm the integration or expression of the *Bt* gene in the putative transgenic plants.

Girijashankar *et al.,* (2005) also attempted to generate transgenic sorghum plants for resistance against the spotted stem borer (*Chilo partellus* Swinhoe). Shoot apices of sorghum genotype BTx 623 were transformed with synthetic *ubi-cry1Ab, ubi-cry1Ac* and *mpiC1-cry1Ac* using particle bombardment method (PIG). Inheritance and expression in T_1 generation for *cry1Ac* gene under the control of the wound-inducible promoter from maize protease inhibitor (*mpiC1*) was confirmed with PCR, RT-PCR and Enzyme linked immuno-sorbant assays. However, the progeny plants of *ubi-cry1Ac* and *ubi-cry1Ab* series showed complete absence of the transgenes. The T_0 transgenic plants generated using *ubi1–cry1Ab* and *ubi1-cry1Ac* were found to be chimeric in nature due to the lack of the transgene *cry* in the reproductive parts.

The germline transmission of the transgenes to their progeny was not detected, while the lower parts of the T_0 generation plants i.e. lower leaves of these plants were transgenic in nature. Insect bioassays (leaf disc assay) at 5 days after infestation indicated that T_1 transgenic plants carrying *mpi-cry1Ac* showed reduction in leaf damage upto 60 % while the neonate larval mortality feeding on the transgenic plants was 40 % and the surviving larvae showed 25 % reduction in weight against the larvae fed on control non-transformed plants. The remaining two series of T_0 transgenic plants carrying *ubi-cry1Ab* (2 plants) and *ubi-cry1Ac* (2 plants) showed low levels of tolerance to the neonate larvae of spotted stem borer (*C. partellus*) while their progeny were completely susceptible as they did not carried the transgenes (Girijashankar, 2005). This was evident from PCR analysis of T_1 generation plants. In response to mechanical wounding, the *cry* gene under the control of wound-inducible promoter (*mpiC1)* expressed 1 - 8 ng of insecticidal crystal protein per gram of fresh leaf tissue at 12 h after wounding (Girijashankar *et al.,* 2005). Whereas, ELISA tests of T_0 plants with *ubi-cry1Ab* and *ubi-cry1Ac* revealed expression of Bt toxin upto 2 and 0.55 ng/gm of leaf tissue, respectively.

Low levels of Bt d-endotoxin expression is reported in transgenic plants among which few plants carrying *mpiC1-cry1Ac* showed partial tolerance against the first instar larvae of *C. partellus,* as mentioned earlier. It was observed that the wound-inducible promoter *mpiC1* was stronger in driving the expression of the transgene *cry* when compared to constitutive promoter *ubiquitin1*. Further, the degree of tolerance towards the shoot fly's neonate larval feeding is in the order of *mpiC1-cry1Ac* > *ubi-cry1Ab* > *ubi-cry1Ac*. This report also forms the first of its kind in transgenic sorghum research where promoter and transgene comparison studies are made on glass house grown plants instead of the regular *in vitro* studies.

Scientists at DSR, Hyderabad, India, reported the production of transgenic sorghum plants using synthetic *Bt* genes *(ubi1-cry1B,* etc). Their aim is to incorporate insect resistance into Indian sorghum hybrids via particle bombardment and *Agrobacterium*-mediated transformation methods. Finally, some of the events are also reported to be promising in insect bioassays because

they showed 80% larval mortality when compared to control non-transformed plants (Visarada *et al.,* 2007; Balakrishna *et al.,* 2007).

Tissue culture and plant chimeras

It is well known fact that *in vitro* regeneration of plant tissues could result in formation of plant chimeras. Chimeras are plants with genetically different cell populations in different cell layers/tissues/organs. Shoot origin studies (Stewart and Dermen 1970; Tian and Marcotrigiano, 1993) have clearly shown that shoots are derived from more than one cell and each descendant of a different apical layer. The later also reported that adventitious shoots can arise from regions where two cell lineages come into contact there by giving rise to chimerical shoots. Christianson (1985) as well as Tian and Marcotrigiano (1993) studied *de novo* shoot meristem formation and observed ephemeral variegated sectors on regenerated shoots. It is concluded that the lower and upper portion of shoot ultimately arise from different group of cells in apical meristem which are formed during *in vitro* cell proliferation.

Lowe *et al.,* (1995) proved that the majority of transgenic sectors were restricted in size in maize explants that were transformed using microprojectile bombardment method. Further, careful manipulation of these transgenic sectors only can increase the likelihood of germline transmission of the transgenes to the next generation. These studies clearly prove the development of chimeric shoots which result in segregation loss of transgenes in their progeny, as untransformed cell lines lead to the formation of reproductive tissues (inflorescence). The germline transformation and chimeric shoot formation depends on the genotype, environmental effects on donor plant, rate of embryo development *in vitro* and *in vivo* and the size of apical dome at the time of bombardment (Lowe *et al.,* 1995).

Girijashankar (2005) reported that molecular analysis and insect bioassays of young leaves of transgenic sorghum plants carrying *ubi1-cry1Ac* and *ubi1-cry1Ab* are transgenic in nature while the same tests with their progeny plants showed the absence and lack of transgene expression. It is hypothesized that the inflorescences and upper parts of the sorghum plants were developed from a group of non-transformed cells while the lower few leaves originated from successfully transformed progenitor cells which could lead to the formation of chimeric sorghum plants. It was thought that chimerism in transgenic sorghum plants was due to mixed *in vitro* regeneration pathways via direct somatic embryogenesis and through intervening callus phase that occurs parallely in the same explant in a given medium (Girijashankar *et al.,* 2007).

Apart from this study, there are no available reports of chimerism in sorghum. Cereal plants with chimeric sectors were generated using direct DNA delivery into shoot apical meristems such as maize (Lusardi *et al.,* 1994). Even, large transgenic sectors that extend through the ear node and into the tassel of mature maize plants failed to produce transgenic progeny (Bowen, 1993).

Transgene silencing phenomenon

Genetic engineering relies on stable integration, desired level of expression and predictable inheritance of the introduced transgene while transgene silencing phenomenon appears to be a major obstacle in the path of transformation efforts in sorghum (Emani *et al.,* 2002). Stable integration and expression of introduced gene is essential to realize transgene advantage in the genetically modified crops. Variation in the candidate gene expression levels are commonly observed in transgenic plants. Once the transgene gets integrated into the genome of the host plant the expression is influenced by the structure, position, epigenetics, silencing, co-suppression and the presence of boundary elements or MARs (Matrix Attachment Regions).

Transgene silencing has been observed in dicotyledons and monocotyledons. Loss of expression is attributed to gene silencing, rather than loss of the transgene in sorghum (Krishnaveni *et al.,* 2004). This occurs through various means which parallels natural gene inactivation mechanisms. Methylation of the introduced DNA and homology-dependent ectopic pairing has been found to be the two major pathways that lead to transgene inactivation (Iyer *et al.,* 2000). During this process, integration intermediates become the targets for DNA methyl transferases that transfer a methyl group to 5' site of cytosine.

In most of the reports on sorghum transformation, it was observed that the introduced *uid*A gene expression was either low or totally absent. Hagio *et al.,* (1991) observed that ß-glucuronidase enzyme activity is very low in sorghum cells compared to other *uid*A gene transformed monocot cells. Their RNA blot analysis revealed accumulation of aberrant transgene mRNA transcripts which the author attributed it to transgene rearrangements and *in vivo* degradation of transcripts. Battraw and Hall (1991) reported that the majority of *uid*A-transformed cells did not stain blue upon incubation with histochemical substrate X-Gluc. Though,

they developed the first successful transgenic sorghum plants using particle bombardment and the plants regenerated were proved to be sterile.

Casas *et al.,* (1993, 1997) noted that GUS activity that is high in initial transient assays could not be detected later (after three weeks of bombardment). They suggested that transgene methylation might have occurred in sorghum cells that inhibited expression of the reported gene. Southern analysis clearly indicated integration of the *uid*A gene, no GUS activity was detected in the T_1 transgenic plants. The *uid*A gene under the control of rice *actin1* promoter (supposed to be target for silencing) has been successfully used in many monocotyledons like rice and it also proved effective in transient expression studies in sorghum.

The final experimental proof for methylation-based gene silencing came from Emani *et al.,* (2002) who reported the absence of GUS activity in the leaf tissues of T_0, T_1 and T_2 generations of transgenic sorghum. They also reported that GUS activity was absent in all tissues tested from regenerated T_0 plants. One possible reason for this may be that the transgenic lines obtained in their investigation had a large number of *uid*A gene copies integrated with possible concatameric arrangements. There are several reports demonstrating transgene inactivation as a result of high copy number integration (Rathore *et al.,* 1993; and Kumpatla *et al.,* 1997). However, results from a number of other studies suggested that transgenes with even low copy number integration could become silenced (Casas *et al.,* 1993; Casas *et al.,* 1997). Similarly, silencing of *uid*A and pathogenesis related genes (*tlp*/*G11*) in other sorghum transformation studies was not attributed to variations in copy number or the method of transformation (particle bombardment or *Agrobacterium*-mediated) (Battraw and Hall, 1991; Jeoung *et al.,* 2004).

Taking the experiment further, Emani *et al.,* (2002) suspected the possible role of the rice *actin* promoter in the silencing of *uid*A gene in sorghum that lead to extremely poor and stable *uid*A transgene expression. This notion was supported by the fact that the *bar* gene that was driven by the *ubiquitin* promoter was expressed well in T_0 and T_1 generations despite the fact that several copies of this gene had integrated into the genome of the same lines showing no GUS activity. It was also observed that during the *in vitro* culture of T_1 and T_2 generation embryos there was spontaneous reactivation of *uid*A gene expression in a small number of calli. Zhu *et al.,* (1998) observed random occurrence of silencing and reactivation of the introduced *chitinase* genes at different growth stages of primary transgenic sorghum plants as well as in their progeny.

5-Azacytidine is a cytidine analog which integrates into DNA during replication or prevents methylation by inhibiting DNA methyl transferase. Emani *et al.,* (2002) reported the reactivation of GUS activity in the azaC-treated tissues and suggested that the silencing of the *uid*A gene resulted from cytosine methylation. It is hypothesized that azaC is incorporated in a small population of rapidly dividing cells; resulting in severe DNA demethylation in a fraction of progenitor cells (Emani *et al.,* 2002).

Further, Emani *et al*., (2002) also observed that the *bar* gene expression under maize *polyubiquitin1* promoter was substantially lower in T_2 progeny when compared to T_0 and T_1 which they tought could be due to the doubling of the high copy number of *bar* genes in homozygous T_1 progeny (Emani *et al.,* 2002). But, a substantial increase in PAT activity in the immature embryo-derived T_2 calli cultures treated with azaC suggested that partial methylation might has reduced the expression of *bar* gene. Krishniveni *et al.,* (2004) studied the loss of rice *chitinase* transgene expression in T_2 and T_3 generation sorghum plants while the *bar* gene present adjacently to it was not silenced. Enhanced *gus* gene expression (upto 70 %) and increased number of transformation spots in sorghum is achieved by flanking the *uid*A transgene on either side by matrix attachment regions (MARs) from tobacco (RB 27 sequences) (Able *et al.,* 2004). Insertions of MARs elements into the gene constructs opens new vistas for generating transgenic sorghum plants and partially address the problem of transgene silencing (especially silencing because of positional insertion).

CONCLUSION

Sorghum is a multipurpose staple crop and the species shows greater diversity. Genetic engineering technology can assist the production of agronomically desirable sorghum plants that exhibit increased resistance to pests, pathogens, abiotic stress and enhanced nutritional qualities. But, few laboratories in the world are addressing sorghum crop improvement through novel methods. So far, limited numbers of genes conferring agronomic advantages have been introduced through *Agrobacterium* and particle bombardment. The most effective method to-date is *Agrobacterium* based sorghum transformation which has high transformation efficiency and is known to produce plants with single copy inserts with complete gene integration.

Promoters such as *ubi1* and *mpiC1* were reported to be the best regulatory elements in sorghum transgene expression. MARS elements play an important and positive role in transgenic expressions by minimizing the positional silencing effect. Rice *chitinase1*, *cry*, *bar*, *HVA I* and *dhdps-raec 1* are the useful genes apart from different marker genes that were transferred into sorghum cultivars, till-date. Development of transgenic sorghum is difficult because of its recalctritant nature along with transgene silencing and chimerism. An efficient tissue culture protocol that follows direct somatic embryogenesis pathway of plant regeneration coupled with MARS elements flanking the candidate gene and *Agrobacterium*-mediated gene transfer method could enable successful introduction and expression of useful genes in sorghum.

Lessons from the recent work by Lu *et al.* (2009) from University of Missouri dealing with the development of marker-free transgenic sorghum plants using *Agrobacterium* co-cultivation strategy should be helpful and can be tried in future. Lu *et al.* (2009) used separate binary vectors containing *bar* and target gene on separate T-DNA regions and co-transformed immature embryo derived calli. Using mild selection pressure (in order not to loose the transformed cells) in T_0 generation followed by evaluating the progeny for transgene segregation, they could finally obtain a fraction of progeny harboring the sorghum *lysyl tRNA synthetase* gene. Thus, they could eliminate the progeny sorghum plants having marker gene co-segregating along with the candidate gene of interest. This line of research could lead the future sorghum transformation to a fruitful result.

Generation and screening of more number of transgenic events should be the prime focus to meet the goal of transformation. Except for few occasions, earlier experiments did not meet these requirements. Instead of working for transformation successes in few T_0 events, the researchers should focus their efforts towards generating multiple events having successful insertion of the transgene into sorghum genome. This can form the platform for the remaining segregation and bioassay studies that can eventually lead towards the successful release of transgenic sorghum plants for the benefit of farmers in arid and semi-arid regions of the world.

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