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Review Article

TISSUE CULTURE, MOLECULAR AND GENETIC APPROACHES TO *SORGHUM* CROP IMPROVEMENT – A REVIEW

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ABSTRACT
Sorghum, a special cereal crop worldwide because of its drought tolerance, is an important staple food in developing countries of the semiarid tropics and is also used as an animal feed. It is also significant for its main source of energy and protein. This article reviews research to establish highly regenerative cell and tissue culture systems in *sorghum* as a prerequisite for crop improvement using biotechnological methods. Various strategies were described based on investigations in tissue culture and new approaches were discussed focusing on the considerable recent progress made using transformation techniques for the enhancement of tissue culture transformation efficiency in *sorghum*. High frequency plant regeneration in *sorghum* still requires standardized protocols using various explants. The review focuses on regeneration response and potential of different explants of *sorghum* and effect of different concentrations of plant growth hormones on them *in vitro*. This review provides an overview of current stage of *sorghum* crop improvement programs, advanced molecular approaches undergoing for crop improvement of *sorghum* by various researchers and their research contributions for its development.

Keywords: *Sorghum bicolor*; *Tissue Culture; Transformation; Molecular Markers*

INTRODUCTION
Genetic improvement of the major cereals such as wheat *(Triticum aestivum),* rice (Oryza sativa), Maize (*Zea mays*), Barley (*Hordeum vulgare*), *Sorghum* (*Sorghum bicolor*), Millet (*Pennisetum* sp), Oat (*Avena sativa*) and Rye (*Secale cereale*) has been particularly important for plant breeders for decades, since these crops provide more than half of the food consumed by mankind being the main sources of plant proteins and carbohydrates. They are also the basis for production of animal feed oil, starch, flour, sugar, alcoholic beverages, renewable energy etc., (FAO, 2007). *Sorghum* is an important food source in Africa and Asia and is widely grown in the southern United States as a cattle feed. In India, *Sorghum* was grown in 7381700 Ha that yielded 9487 Hg/Ha and recorded as top producer of *Sorghum* in the world. *Sorghum* ranks fifth in India for commodity value (FAO STAT, 2011). In India, *Sorghum* or Jowar is predominantly grown in the arid and semi arid regions like Maharashtra, Andhra Pradesh, Karnataka and Tamilnadu areas with as little as 400 to 500mm rainfall per year. To ensure the world's food supply for the future with a continued population growth up to 8 billion people in 2025 will demand for cereals to increase by 41% between 1993 and 2020 to reach 2.490 million metric tons (Dyson, 1999; Pinstrup–Anderson, 2001). To meet the dramatic increase in cereal demand worldwide, new approaches and technologies for generating new varieties are necessary. The rapidly developed methods of molecular and genetic engineering provide powerful and novel means to supplement and complement the traditional methods and categorized as alternative approaches for crop improvement. Inspite of the molecular advances, transformation efficiency in *sorghum* is less due to limitations like genotype, explants type and short regeneration ability. *Sorghum* has been categorized as one of the most difficult plant species for Tissue culture and transformation (Zhu *et al*., 1998). The application of gene transfer to improve traits in a desired cultivar is strongly limited in *sorghum*, because a highly efficient and reproducible regeneration system is only available for a few so- called model genotypes. Considerable progress has been made concerning invitro regeneration of cereals and grasses during the last decades screening genotypes worldwide, various explants sources and numerous media constituents. Inspite of these advances, the

number of highly responsive genotypes suitable for genetic transformation experiments is still limited due to extensive genotypic variation for tissue culture performance (Jutta, 2007). The expected ability of young leaf tissues of gramineceous species to express morphogenic capacity was first demonstrated for *sorghum* (Wernicke and Brettell, 1980). First successful plant regeneration using immature inflorescence of *sorghum* was reported by Brettel *et al*., (1980). The first report of successful transformation of *sorghum* appeared as early as the 1990's. Efforts are in progress to transfer genes mtlD, p5CSf129A, Cod A to Indian *sorghum* genotypes for biosynthesis of osmoprotectants. Expression of these genes leads to accumulation of osmolytes resulting in tolerance to various abiotic stresses. The empowerment of standardized protocols for gene transformation and regeneration in *sorghum* opens up new opportunities to improve protein nutritional quality, high yield and drought resistant cultivars which serve as an ideal staple food for ever increasing population. Inculcating agronomically important traits such as disease resistant, salt tolerant, drought tolerant, insect-pest resistant, herbicide resistant, high-yielding and high nutritive value into cultivating crops grasps interest and gains attention for its improvement, utilization and cultivation. The ultimate aims of genetic transformation studies are to develop user friendly vector system applicable to a wide range of species. Microprojectile bombardment employs high velocity metal particles to deliver biologically active DNA into plant cells (Sanford, 1998). Frequency of transient to stable transformation events is very low in *sorghum* transformation. SSR (Simple Sequence Repeats) Markers are found to be the most popular markers for crop improvement of *sorghum* in respect to its transferability, genetic diversity studies, QTL Mapping and Marker assisted crop breeding (Nagaraja *et al*., 2011) Mining of SSR markers from ESTs has been reported in *sorghum* by (Srinivas *et al*., 2008).

Tissue Culture and Transformation Studies

Advances in Biotechnology are applied to augment traditional approaches for crop improvement. Methods of plant transformation for transgenics require genetically transformed whole plant that is regenerated from isolated plant cells or tissue through regeneration invitro. The ultimate aim is high frequency of regeneration, accessibility to gene transfer and transformation efficiency. High frequency plant regeneration from cultured explant material is a prerequisite for successful transformation of most cereal crops. Cereal crop improvement through genetic transformation requires establishment of an efficient and reproducible plant regeneration system and it is also essential for complete transformation protocol (Jha *et al.,* 2009). Progress in *sorghum* transformation has been hampered by difficulties associated with tissue culture, such as accumulation of phenolic pigments and low regeneration frequencies. The long periods of selection needed for the recovery and regeneration of putative transgenic plants often hampered optimization of conditions for *sorghum* transformation. Probably, low transferability of *sorghum* was predicted as occurrence of DNA methylation in *sorghum* cells that inactivates the expression of transferred genes.

The first experiments to culture plant cells under invitro conditions were conducted years ago (Haberlandt, 1902). However in *Sorghum*, the earliest work on *in-vitro* cultured was reported by Strogonov *et al.,* (1968), they reported callus induction from aseptically germinated *Sorghum* seedlings. Masteller and Holden (1970) reported that, the callus growth may be the growth of aberrant meristematic tissue and not undifferentiated cells. They also showed that, this callus growth generally forms at the basal node of the *sorghum* seedlings in response to 2, 4 D an auxin analogs and the growth regulator of choice. Gamborg *et al*., (1977) observed morphogenesis and plant regeneration from callus cultures of immature embryo of *sorghum*. They reported that, cultured explants released black and purple pigmented material into the medium, which causes the growth retardation of callus cultures. They also observed somaclonal variations i.e., variation in leaf morphology and growth habit. This was the first report of somaclonal variation from cell and tissue culture derived cultures of *sorghum*. There have been reports of regenerated *sorghum* plants with useful traits such as male sterility and disease resistance. Somaclones of *sorghum* have been produced with tolerance to high concentrations of salts like aluminum and manganese (Smith *et al*., 1983; Bhaskaran *et al*., 1985; Mgema and Clark, 1995) and tolerance to acid soils (Waskom *et al*., 1990; Duncan *et al*., 1991b, 1995; Miller *et al*., 1992; Foy *et al*., 1993). Smith *et al*., (1982) and Duncan *et al*., (1995) reported drought tolerant somaclonal variants. Pest-resistant Somaclones have also

been reported (Isenhour *et al*., 1991). Isenhour *et al.,* (1991) found that tissue culture derived *Sorghum* plants exhibits resistance to leaf-feeding by the fall Armyworm. They reported that, tissue culture induced variations can be a viable means of generating new sources of genetic diversity for use in crop improvement. Jeoung *et al.,* (2002a) reported optimization of *Sorghum* transformation parameters for both the *Agrobacterium* and Biolistic bombardment methods. Transient *Gus* expression in cultured shoot tips of *Sorghum* was observed by Devi *et al.,* (2001) they developed an optimal micro projectile bombardment procedure for *Sorghum*. Adventitious shoot regeneration from immature embryos of *Sorghum* was reported by Hagio (2002). He used 11 genotypes of *Sorghum* for their response in tissue culture. He also observed the position effect of proline and PVP on shoot formation. Direct somatic embryogenesis from isolated shoot apex was reported by Harshavardhan *et al.,* (2002). They developed an improved protocol for direct somatic embryogenesis by using MS+5µM of TDZ + 17.72µM BAP + 1.074µM NAA, for root induction they used 8.28 µM of IBA and 1.14 µM IAA.

Visarada *et al.,* (2003) reported that, tissue culture protocols are genotype specific and suitable protocols need to be developed when a new variety is to be used. They made a detailed study on callus induction and regeneration using different explants of *Sorghum*. They reported multiple shoot induction and regeneration using 1-6 mg/L concentration of BAP. Different explant sources like mature embryo, immature embryo, immature inflorescence, shoot tip, leaf base were used to check tissue culture response and were trailed for transformation procedures. Immature embryos have been shown to be the most successful and productive explants for *Sorghum* tissue culture (Elkonin and Pakhomova, 2000; Grootboom *et al.,* 2010; Gurel *et al.,* 2009). Anjuverma and Anandkumar (2005) developed an efficient plant regeneration system from different explants of *Sorghum;* they reported multiple shoot induction by using 2 mg/L of BAP in the culture medium. Mature seeds are the most preferred explants for invitro protocol studies as they can be stored, available round the year and can easily handled (Kishore *et al.,* 2006) but, Mature embryo and leaf bases resulted in limited callus initiation (Motl and Cure, 1998). Grootboom *et al.,* (2008) worked on invitro studies on *sorghum* using immature embryos. Their statistical analysis of the data showed that the response to both callus induction and regeneration were influenced by medium and genotype independently. A study was conducted by Sudhakar *et al.,* (2007) in our laboratory on effect of various plant growth hormones on *sorghum* tissue regeneration using immature embryo as explant source. The results depicted that highest callus induction frequency was observed using combination of 2, 4-D and KN at the concentration of 2mg/l+0,5mg/l.

Various Gene transfer methods such as *Agrobacterium* mediated, Microprojectile mediated, Particle Bombardment and Biolistic gun were done and screened for transformation efficiency. *Sorghum* transformation has been widely considered as challenging since the first transgenic *sorghum* was reported in 1993 (Casas *et al.,* 1993) by biolistic bombardment. Transformation report by *Agrobacterium* mediated was successfully done by Zhao *et al.,* (2000) (Table 2). These two reports are considered as pioneer works in Sorghum transformation studies.

Battraw and Hall (1991) reported the first genetic transformation of *sorghum* protoplasts with chimeric neomycin phosphotransferase II and β-glucuronidase (*gus*) genes by electroporation, but failed to achieve plant regeneration. *Agrobacterium* mediated transformation systems take advantage of its natural plant transformation mechanism (Trends in plant science 2000). *Agrobacterium* mediated transformation (Herella – Estrella, 1983) has become the most used method for the introduction of foreign gene into plant cells and the subsequent generation of transgenic plant due to its simplicity and its efficiency in expessebility of transformed genes. The first report of *Agrobacterium* mediated transformation was given by DeBlock *et al.,* (1984).

Microprojectile transformation and *Agrobacterium* mediated transformation are the two main approaches that have been utilized to obtain transgenic *sorghum*. Bombardment transformation efficiency is determined by physical, biological and environmental factors such as the concentration of microparticles and DNA, conditions of acceleration, target distance, pre and post-bombardment culture conditions, the type and physiological condition of the explant and the choice of selectable marker gene and a strong gene promoter (Tadesse *et al*., 2003). The attempt of insertion of disease resistance trait in *Sorghum*

against Stalk rot by using rice chitinase gene was succeeded by Krishnaveni *et* al., (2001). Emani *et al.,* (2002) made use of the cytidine analog, 5-azacytidine, in reversing the methylation mediated *gus* gene silencing. Shridhar *et al.,* (2010) worked on *Agrobacterium* mediated transformation studies in *sorghum* using *gfp* reporter gene they summarized that maximum callus induction frequency was obtained with immature inflorescence (81.9%) followed by seedling tissue (61.0%). There are some reports describing the successful biolistic transformation of *sorghum* plants with marker genes and insect resistant genes (Hagio *et al*., 1991; Casas *et al*., 1993, 1995, 1997; Kononowicz *et al*., 1995; Hagio, 1998; Zhu *et al*., 1998). Tadesse *et al*., (2003) optimized microparticle bombardment transformation conditions and marginally increased transformation efficiency (1.3%). The report of Zhao *et al.,* (2003) which resulted transgenic *Sorghum* with improved protein nutritional quality made a significant path. Carvalho *et al*., (2004) developed three transgenic *sorghum* events through the use of a ‘‘super-binary’’ vector with *hpt* (hygromycin phosphotransferase gene) as a selectable marker.

GirijaShankar *et al*., (2005) reported successful recovery of transgenic *sorghum* plants, with the transgene *cryIac* expressed under the control of the wound inducible promoter *mpiCI* from maize, by particle bombardment of shoot apices with a transformation efficiency of 1.5%. A. Raghuwanshi and Birch (2010) reported the first particle bombardment mediated transformation of sweet *sorghum* with transformation efficiency of 0.09%. Transgenic *Sorghum* using chitinase and chitosinase genes for insect resistance against *Chilo partellus* was reported by Kosambo-Ayoo (2012). Recently, Guoquanliu and Ian Godwin (2012) reported that the enhancement of *Sorghum* transformation efficiency can be largely attributed to three crucial factors: i) Tissue culture system ii) DNA delivery system iii) Selection strategy. They also reported that a highly efficient gene transfer system largely resets on an effective tissue culture system and an optimal DNA delivery system. They obtained highest transformation efficiency of 20.7%, using Microprojectile bombardment. Standardization and generating efficient transformation protocols in *sorghum* facilitates enhanced implementation of molecular approach for its crop improvement, further, provides pathways for applying genetic engineering strategies and thus place *sorghum* as a model plant for Cereal research. Thus, Tissue culture system plays a fundamental role in the success of *Sorghum* transformation system. Factors that majorly influence effectiveness of *sorghum* tissue culture are: i) Explant source ii) Genotype iii) Composition of the medium (Callus induction medium; Regeneration medium; Rooting medium). *Invitro* cultures of *sorghum* also show strong genotype dependence (Jogeshwar *et al*., 2007).

The success and utility of plant transformation protocols described above depend very much on the levels of expression of the introduced genes Promoters and reported genes also play an important role in optimizing DNA delivery system (Guoquanliu and Ian, 2012). 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). *GFP* is a widely used reporter gene (construct) in *Sorghum* transformation. It produces a protein that fluoresces in living cells when exposed to blue light at the wavelength of 395nm. Chowdhury *et al.,* (1997) assessed the efficiency of five commonly used promoters (for monocotyledonous species) including Adh1 (Ellis *et al*., 1987), Ubiquitin, Actin1, Emu and CaMV 35S. Their results indicated that the Emu or Ubiquitin promoter would be the most reliable in developing constructs suitable for high level expression of transgenes in oil palm.

β-glucuronidase (*GUS*) gene (Jefferson *et al.,* 1987), encoded by the *uidA* locus of *Escherichia coli*, that can be readily evaluated by histochemical as well as fluorometric assays (Cho *et al.,* 1999). The R gene of maize, which regulates the anthocyanin biosynthesis, requires no external substrate but produces distinct pigmentation in cells in which it is expressed (Ludwig *et al*., 1990); it was found to be successful to study the gene expression in maize. The green fluorescent protein coding gene (*gfp*) from jelly fish) Chalfie *et al.,* (1994) has been attracting significant attention as a more useful marker than *GUS*, since its assay is

more simple, non destructive and requires no external substrate (Tyagi *et al.,* 1999; Chung *et al.,* 2000). Efficient transgene expression requires presence of suitable promoter and a terminator. As integrative transformation occurs at low frequency, efficient production of transgenic plants requires a careful choice of an appropriate selectable marker to distinguish transformed and untransformed plant cells. In genetic transformation experiments, selectable markers allow identification of transformed cells based on selective growth of the transformants, when grown on medium containing the selection agents. Accordingly, most of the strategies for the selection of the rare transformed cells are based on selective inhibition of the growth of untransformed or wild-type cells, without significant affect on the transformed cells (Vasil, 1994). This has been achieved by introducing a gene for antibiotic/drug/herbicide resistance under the control of a constitutive promoter like the cauliflower mosaic virus (CaMV) 35S promoter, or monocot promoters with high constitutive activity, such as maize *Ubi1* and rice *Act1*.The cauliflower mosaic virus (CaMV) 35S has been most commonly employed in transformation of dicots as well as monocots for high and constitutive expression. But in view of the relatively low activity (100-fold less than in dicots) of this promoter in monocots, the other promoters have been tested (Fromm *et al.,* 1985; Hauptmann *et al.,* 1987).

Table 1: Studies on different explant sources for *sorghum* Tissue culture *in vitro*

Explant Source	References	Regeneration studies
Apical meristem or shoot apex	Seetharama *et al*., 2000; Nahdi and dewet 1995	Plant regeneration
Immature embryos	Sato.S *et al.,* 2004; Sudhakarpola *et al*., 2007; Cai and Butler 1990; Rao *et al.,* 1995; Rathus *et al.,* 1996 ; Hagio 2002 ; Visarada *et al.,* 2003 ; Gamborg *et al.,* 1977 ; Dunstan *et al.,* 1978 ; Ma and Liang, 1987 ; Groot boom *et al*., 2008 ; Elhag *et al.,* 1992 ; Sudhakar pola *et al.*,2009	Morphogenesis and plant regeneration; Varietal differences in regeneration; Multiple shoot induction; Long term maintenance of callus cultures
Mature seeds	Murthy *et al.,* 1990; Hagio *et al.,* 1994 ; Visarada *et al.,* 2003 ; Sudhakar pola *et al.,* 2009	Callus induction and effect of PGR's on regeneration
Immature inflorescence	Thomas *et al.,* 1977 ; Brettell *et al.,* 1980 ; Arti *et al.,* 1994 ; Casas *et al.,* 1997 ; Rao *et al.,* 2000 ; Visarada *et al.,* 2003 ; N.Saradamani *et al.,* 2003; Sanjay Gupta *et al*., 2006; Jogeshwar *et al.,* 2007	Embryogenesis; Somatic embryogenesis and plant regeneration; Strategies to overcome genotypic limitations of *invitro* regeneration
Leaf discs	Wernicke and Brettell 1982; Cai *et al.,* 1987; Bhaskaran *et al.,* 1989 ; Elkonin *et al.,* 1993 ; Anjuverma and Kumar, 2005; Sudhakarpola 2011	Callus induction and Regeneration
Seedlings	Strogonov *et al.,* 1968; Masteller and Holden, 1970; Smith *et al.,* 1983; Kresovich *et al.,* 1986	Callus induction and regeneration
Shoot tip	Bhaskaran *et al.,* 1988; Bhaskaran and Smith, 1990; Zhong *et al.,* 1998; Seetharama *et al*., 2000; Prathibha and Sticklen 2001; Harshavardhan *et al.,* 2002 ; Saikishore N *et al*., 2006 ;	Embryogenesis and regeneration studies; invitro culture methods and field performance of progeny
Leaf segments	Sudhakar Rao pola and Sarada Mani 2006	*Invitro* plant regeneration and somatic embryogenesis

Table 2: Approaches used for Transformation with regeneration of *Sorghum bicolor L. Moench*

Explant Source	Genotype	Trans genes	Method of transformation	Transformation efficiency/studies	References
Protoplasts	---	*cat*	Electrop-oration	Efficient gene expression	Ou-Lee *et al.*, 1986
Cell Suspensions/ Protoplasts	---	*npt II*	Electrop-oration	Stable transformation	Battraw and Hall, 1991
Cell suspension culture	---	*npt II, hpt, udi A*	PDS-1000/ He (Bio-Rad)	Stable transformation	Hagio *et al.*, 1991
Immature embryos	---	*bar, udi A*	PDS-1000/ He (Bio-Rad)	Plants regeneration at low frequency	Casas *et al.*, 1993
Immature embryo/ inflorescence callus	---	*bar, udi A & luc*	PDS-1000/ He	Plants regeneration at low frequency	Kononowicz *et al.*, 1995
Immature embryo/inflorescence callus	---	*bar*	Particle inflow gun	Single plant reported	Rathus *et al.*, 1996
Immature inflorescence	P898012	*gus* and *bar* genes	Microproj ectile bombardment	0.286%	Casas *et al.*, 1997
Immature embryos	---	*bar/Chitinase I*	PDS-1000/ He	----	Zhu *et al.*, 1998
Immature embryo	---	*bar* gene using CaMV 35S/ *act 1* as promoter	Particle inflow gun	Used Casein hydrolysate to enhance regeneration frequency	Rathus and Godwin 2000
Immature embryos	P898012; PHI391 (Commercial line)	*bar* gene for herbicide resistance	*Agrobacterium* mediated	2.1%	Zhao *et al.*, 2000
Immature embryos callus	---	*uid A*	Particle bombardment	Promoter studies	Hill-Ambroz and Week 2001
Leaf and calli	The Indian cultivar, M35-1; a Hegari-type, SA281; an Australian inbred line, QL41;	The *uid A* reporter gene *GUS* ; *GFP* used for screening later pAHC20 construct (Containing	Particle inflow gun	Optimized Parameters for transgene expression	Able *et al.*, 2001

	and an American inbred line, P898012)	the bar gene) was used			
Immature embryo callus	---	*uid A, bar* using *act I and ubi 1* as promoters	Particle bombardment	Methylation based transgene silencing	Emani *et al.*, ,2002
Immature zygotic embryos; 7-10 day old embryogenic calli; leaves	Tx 430, C401 (a Chinese inbred); CO25	*GFP* and glucuronidase (*gus*) as reporter genes *ubi*-1 as promoter	*Agrobacterium* mediated and Biolistic bombardment	Reported *gfp* to be superior to *gus*. Optimized transformation conditions	J.M. Jeoung *et al.*, 2002
Immature and mature embryos	The accession no. '214856' from Ethiopia	*dhdps raec I* mutated gene; *uidA* as reporter gene	Microprojectile bombardment	1.3%	Tadesse *et al.*, 2003
Shoot meristems	---	*bar/HVA I* using CaMV 35 s promoter	PDS-1000/He	On Drought tolerance	Devi *et al.*, 2004
Shoot meristems	---	*bar, cry I Ab & cry I B*	Particle inflow gun	On insect resistance	Gray *et al.*, 2004
Immature embryos/Shoot tips	---	*nptII, dhdps-raec I*	PDS-1000/He	Promoter studies	Tadesse and Jacobs 2004
Immature embryo callus	---	*hpt, npt, uid A*	*Agrobacterium* mediated	---	Carvalho *et al., 2004*
Immature embryo callus	---	*gfp/bar/tlp/rice chitinase* GII using *ubi 1* promoter	*Agrobacterium* mediated	---	Jeoung *et al.*, 2004
Shoot apices	BTx623	*Cry 1 Ac gene, bar, Uid A gene* using *mpi CI* as promoter	Co-bombardment particle inflow	1.5%	GirijaShankar *et al.*, 2005
Immature embryo Callus	---	*gfp/tlp*	*Agrobacterium* mediated	Marker free studies and southern blot for *tlp* reported	Gao *et al.*, 2005
Immature embryo callus	Tx430; C401 and a commercial hybrid	Dual marker plasmid containing *manA* and *pmi* gene	*Agrobacterium* mediated	2.88% and 3.30%	Gao *et al.*, 2005a

	Pioneer 8580	phosphomannose isomerase as selectable marker and *gfp* as reporter gene			
Immature embryos	Tx430; C2-97	*Agrobacterium* mediated	Non herbicide resistance gene (*npt II)* as selectable marker	0.3-4.5%	Howe *et al.*, 2006
Immature embryo derived calli	Red *sorghum* cultivar Sensako 85/1191 (Monsanto, South Africa)	*npt II* as selectable marker gene (hygromycin phosphotransferase gene)	*Agrobacterium* mediated	5%	Nguyen *et al.*, 2007
Pollen	---	*npt II, uid* A	Mild Ultra Sonication	PCR and Southern Blot reported	Wang *etal.*, 2007
Heat treated Immature embryos	P898012	phosphomannose isomerase (*PMI*) as selectable marker; *gfp* as reporter gene	*Agrobacterium* mediated	49.1% *GFP*-expressing calli and 8.3% stable transformation frequency	Gurel *et al*., 2009
Immature embryos	P898012	High lysine protein gene *HTL 2; bar* gene as a plant selectable marker and the *GUS*-intron gene as a reporter	*Agrobacterium* mediated	0.4 and 0.7% Marker free transgenic *sorghum* studies	Lu *et al.*, 2009
Immature zygotic embryos	P898012	*bar* gene and *man A* for phosphomannose isomerase	Particle bombardment	0.77%	A. W. Grootboom *et al.*, 2010
Immature embryo	Ramada (Sweet *Sorghum*)	*hpt* , *Luc*	Microprojectile bombardment	0.09%	Anshu Raghuwanshi and Robert G Birch, 2010
Immature zygotic	Kat 412;	Chitinase and	Particle Bombardment	Enhanced	Linus Moses

embryos	Serena; KAT 487; SDSH 513; GBK046820; ICSV; KAT L5	Chitosinase		transformation efficiency.	Kosambo-Ayoo *et al.*, 2011
Immature embryos	P898012; RTx430	*GUS* gene under the control of CaMV 35 S Promoter	*Agrobacterium* mediated	Observed 2.9 fold increase in transformation efficiency using l-cysteine in the medium during Co-cultivation step.	Vinod kumar *et al.*, 2011
Immature seeds	Tx430	*npt* II; *gfp*	Microprojectile bombardment	20.7%	Guoquanliu Ian D Godwin, 2012

cat: Chloremphenicol acetyl transferase; hpt: Hygromycin phosphotransferase; luc: Luciferase; gfp: green fluorescent protein; CaMV35S: cauliflower mosaic virus promoter; nptII: neomycin phosphotransferase II; Adh1: maize alcohol dehydrogenase gene promoter; gus or uidA: β-glucuronidase gene

Table 3: Molecular approaches for crop improvement using various molecular markers

RFLPs	SNPs	RAPDs	AFLPs	SSRs	STA
Restriction length polymorphism Use: Construct gene maps; Molecular tagging of various agronomic traits.	**Single nucleotide polymorphism** Use: Ease of notifying in all parts of the genome.	**Random amplified polymorphic DNAs** Use: Construct gene maps; Molecular tagging of various agronomic traits.	**Amplified fragment length polymorphism** Use: Selective amplification of restriction fragments giving rise to large number of useful markers.	**Simple sequence repeats or Microsatellites** Use: Determine the degree of relatedness among individuals.	**Sequence tagged sites** Use: Characterizing and identification of genetic resources.

Plasmid constructs based on the maize ubiquitin promoter have been shown to provide the highest levels of gene expression in several species (Carnejo *et al.*, 1993; Taylor *et al.*, 1993) and have been used to obtain transgenic plants of rice, wheat and barley (Toki *et al.*, 1992; Vasil *et al.*, 1993, Wan and Lemaux 1994). Constructs with rice *Adh1* have been similarly used to obtain transgenic rice (Zhang *et al.*, 1991) and *sorghum* (Battraw and Hall, 1991; Hagio *et al.*, 1991; Casas *et al.*, 1993; Tadesse and Jacob, 1995). The Ubi I promoter has proven to be the most successful promoter in *sorghum* transformation (Groot *et*

al., 2010; Raghuwanshi and Birch, 2010; Gurel *et al*., 2009). Strength of promoters used for *sorghum* transformation in descending order: Ubi I > act I D > adh I > CamV 36 s (Tadesse *et al*., 2003).

Molecular Studies

Sorghum Genome Sequencing

Deciphering genome sequence of *sorghum* in 2009 by Andrew *et al*., using whole genome shotgun sequence was a land mark scientific achievement in *sorghum* research. *Sorghum* comprises small genome (~730 Mb) that makes an attractive model for functional genomics. The true worth of plant genome information lies in translating those data into an improvement of crops through various breeding strategies. Gene discovery and functional identification for the predicted genes using functional genomics provides the genomics resources for crop improvement (Shridhar *et al*., 2010). Molecular markers are identifiable DNA sequences found at specific locations of genome and transmitted by the standard laws of inheritance from one generation to the next. They should not be considered as normal genes, as they usually do not have any biological effect and instead can be thought of as constant landmarks in the genome, thus also used to develop gene tags. Recent molecular advances in crop improvement include introgression of Qualitative trait loci for disease resistance, high yield and other important traits from wild relatives and related species through wide crosses (Caius, 2004). Marker assisted studies are useful for assessing Functional diversity, Transferability and Comparitive mapping. Application of *Sorghum* markers in other cereals offer an opportunity for using them in a variety of studies such as flanking markers for synteny based targeted mapping of QTL in less studied crops. Numerous SSR markers have been developed and mapped for *sorghum* (Taramino *et al*., 1997; Schloss *et al*., 2002). Nagaraja *et al.*, (2011) reported high transferability rate of *Sorghum* bicolor markers into its wild species indicating that all bicolor derived markers can be readily applied in *sorghum* wild species to link and introgress useful genes and traits into cultivated *sorghum*s. RFLP linkage maps are being constructed that should greatly facilitate plant breeding efforts for marker assisted backcross programs (Whitcus, 1992) and also used to clone agriculturally important genes through the use of map based cloning strategies (Martin *et al.*, 1993). Remarkable data have been accumulated revealing that tissue culture ability is under genetic control. SSR or microsatellite markers have been developed more recently for crop plants and they are regarded as promising marker system which is applicable for marker development and implementation in breeding programs. Agrama *et al.*, (2003) worked on Phylogenetic diversity and relationships among 22 *sorghum* genotypes with important agronomic traits using SSRs and RAPDs. The results revealed that SSR markers were highly polymorphic than RAPD primers. At the outset, they remarked SSR markers to be useful for the estimation of genetic similarity among diverse genotypes of *sorghum*.

Molecular markers major application is marker assisted selection, which is promising, relatively simple and can be automated. They also assist and support the selection of lines with the desired characteristics leading to the production of improved *sorghum* varieties. Wendy *et al*., (2004) used molecular markers for the selection of *sorghum* crops with lower level of amylose, an enzyme present in the food grain that restricts the processivity of *sorghum* product in cattle feed. By using molecular markers we can test the presence of specific genes or characters in the absence of the pest or the stressor of reduced yield. Marker assisted selection allows plant selection at the juvenile stage from an early generation (Viktor, 2000). Further research for molecular marker development in related to *sorghum* crop is needed because; there is insufficient quality of markers, inadequate experimental design to upgrade complex quantitative traits. Molecular markers have been used to identify and Characterize QTL associated with several different traits in *sorghum* including plant height and maturity (Pereiva and Lee, 1995), plant domestication (Patterson *et al.*, 1995), disease resistance (Gowda *et al.*, 1995), drought tolerance (Tuinstra *et al.*, 1996, 1997, 1998). Molecular marker technology offers a possibility by adopting a wide range of novel approaches to improving the selection strategies in Cereal breeding (Victor, 2000). Agrama *et al.*, (2003) worked on Phylogenetic diversity and relationships among 22 *sorghum* genotypes focusing important agronomic traits using SSRs and RAPDs. The results revealed that SSR markers were highly polymorphic than RAPD primers. At the outset, they remarked SSR markers to be useful for estimation of genetic similarity among diverse genotypes of *sorghum*. Remarkable applications of SSR markers are: i) Co

dominant and highly informative. ii) Display high levels of polymorphism. iii) Amenable to automated genotyping strategies. iv) Radio isotopes are not required in the detection of SSR markers, because, sequence polymorphism can be detected by separation in agarose gels (Burr, 1994). Gowda *et al.,* (1995) and identified DNA markers for downy mildew-resistant genes. Oh *et al.*, (1996) reported tagging of Acremonium wilt, downy mildew and head smut resistance genes in *sorghum* using RFLP and RAPD markers. Brown *et al.,* (1996); Taramino *et al.,* (1997) identified SSR markers in *sorghum*. Ejeta *et al.,* (2011) worked on the genetic and physiological mechanisms that condition drought tolerance in *sorghum*. They found QTL mapping analysis to be promising to analyze the link between the expression of stay green under post flowering drought and grain yield under non-drought conditions. They also designed molecular map for striga resistance genes and generated a fairly dence linkage map of *sorghum*. Single marker analysis detected six QTL for resistance to S.hermothica and five QTL for resistance to S.cisiatica Marker development for drought tolerance from *sorghum* can be done through carefully monitoring, characterizing appropriate germplasm under stress conditions. Several SSR loci were characterized and tested on inbred lines of *sorghum*. Recently, Satish *et al*., (2012) developed 80 Candidate gene (CG)-based markers targeting the seven most important shoot fly resistance genomic regions. They genetically map the candidate genes of *sorghum* based on microsatellite and intron polymorphisms, to identify their significant allelic association with Shootfly resistance through MQM analysis using a recombinant inbred population (RIL).

Applications of Molecular Markers in Sorghum Research

- Finger printing of elite genetic stocks.
- Assessment of genetic diversity.
- Increasing the efficiency of selection for difficult traits.
- Makes environment neutral selection possible.
- Selection for desirable genotypes.
- Manipulation of qualitative test loci that condition complex economic traits.
- Correctly map or place the various interacting genes that condition complex agronomic traits that are inturn useful for effective manipulation of imported genes.

Aridization of climate in many regions all over the globe hampers sustainable production of cereals that are primary food sources and also suspects' food security for future generations. *Sorghum* being a high productive, low input, heat tolerant and drought resistant crop attains global attention for its specificity. Developing a transformation system for *sorghum* is compounded by the difficulties associated with acclimatizing invitro plants into soil which leads to losses of any stable transgenics regenerated (Sai *et al*., 2006).

Different strategies to establish standardized protocols for efficient regeneration system and from that effective transformation protocol are very essential for transgenics in *sorghum*. Tissue culture, that covers all aspects of the cultivation and maintenance of any plant material invitro, is an essential study successful transformation. Genetic manipulation must instill novel traits in elite breeding lines of *sorghum* that targets the objectives like disease - pest resistance, drought and salinity tolerance and improvement of the quality of the grain. Comprehensive review of the published literature on *sorghum* tissue culture and transformation revealed that transformation efficiency can be enhanced by improving standardized regeneration protocols. Over the past decade genomics recourses available for *sorghum* have rapidly expanded (Paterson *et al*., 2008).

Integration of the *sorghum* genetic map developed from QTL information with the physical map will greatly facilitate the map based cloning and precise dissection of complex traits such as drought tolerance in *sorghum*. More research is needed in the area of *sorghum* DNA based maps for identifying and characterizing genes of interest. Tools of biotechnology provide great potential for the exploitation of untapped germplasm of *sorghum*. The research advances in genomics will greatly accelerate the acquisition of knowledge with further development of tools for modifying and interrogating genomes. ICRISAT developed diversification of *sorghum* breeding programs by the incorporation of new traits and genetic materials. An effort to insert pest resistance in *sorghum* seems to be successful for Shootfly and

Midge. Contributions of National science foundation under Plant Genome Research significantly delivered research advances in *sorghum* focusing on discovering the function of genes. The twenty-first century was marked by dramatic advances in scientific approach and comprehensive understanding and deciphering of function of genes at the molecular level. DNA manipulation for more productive and environmental friendly agriculture by successfully using technologies like tissue culture and transformation greatly contributed for mankind. Genomics has opened up new perspectives and opportunities for marker assisted selection for plant breeders, to assess and enhance diversity in their germplasm collections, to introgress valuable traits from new sources and to identify genes that control key traits.

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