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

*Sharmila Polumahanthi1, Sarada Mani N.1, Sudhakar Pola2, Dora S.V.V.S.N.1, and Nageswara Rao S.1
1Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India
2Department of Biotechnology, Andhra University, Visakhapatnam, Andhra Pradesh, India
*Author for Correspondence

ABSTRACT

Sorghum, a special cereal crop worldwide because of its drought tolerance, is an important staple food in developing countries of the semi-arid 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 Tamil Nadu areas with as little as 400 to 500 mm 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 gramineaceous 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 osmoproducts. 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. Microparticle 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 (Ishenhour et al., 1991). Ishenhour 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 (Herrella – 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 expresseeability 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
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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. Giritia Shankar et al., (2005) reported successful recovery of transgenic sorghum plants, with the transgene crylac 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. Raghuvanshi 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 manA 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

<table>
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<tr>
<th>Explant Source</th>
<th>References</th>
<th>Regeneration studies</th>
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<tr>
<td>Apical meristem or shoot apex</td>
<td>Seetharama et al., 2000; Nahdi and dewet 1995; Sato.S et al., 2004;</td>
<td>Plant regeneration</td>
</tr>
<tr>
<td></td>
<td>Cai and Butler 1990; Rao et al., 1995; Rathus et al., 1996; Hagio 2002;</td>
<td>Morphogenesis and plant regeneration; Varietal differences in regeneration; Multiple shoot induction; Long term maintenance of callus cultures</td>
</tr>
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<td></td>
<td>Visarada et al., 2003;</td>
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<tr>
<td>Immature embryos</td>
<td>Gemtorg et al., 1987; Dunstan et al., 1978; Ma and Liang, 1987; Groot boom et al., 2008; Elhag et al., 1992; Visarada et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Mature seeds</td>
<td>Murthy et al., 1990; Hagio et al., 1994; Visarada et al., 2003; Sudhakar pola et al., 2009</td>
<td>Callus induction and effect of PGR’s on regeneration</td>
</tr>
<tr>
<td>Immature inflorescence</td>
<td>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</td>
<td>Embryogenesis; Somatic embryogenesis and plant regeneration; Strategies to overcome genotypic limitations of invitro regeneration</td>
</tr>
<tr>
<td>Leaf discs</td>
<td>Wernicke and Brettell 1982; Cai et al., 1987; Bhaskaran et al., 1989; Elkonin et al., 1993; Anjuverma and Kumar, 2005; Sudhakarpola 2011</td>
<td>Callus induction and Regeneration</td>
</tr>
<tr>
<td>Seedlings</td>
<td>Strogonov et al., 1968; Masteller and Holden, 1970; Smith et al., 1983; Kresovich et al., 1986</td>
<td>Callus induction and regeneration</td>
</tr>
<tr>
<td>Shoot tip</td>
<td>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</td>
<td>Embryogenesis and regeneration studies; invitro culture methods and field performance of progeny</td>
</tr>
<tr>
<td>Leaf segments</td>
<td>Sudhakar Rao pola and Sarada Mani 2006</td>
<td><em>In vitro</em> plant regeneration and somatic embryogenesis</td>
</tr>
</tbody>
</table>
Table 2: Approaches used for Transformation with regeneration of *Sorghum bicolor* L. *Moench*  

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<tr>
<th>Explant Source</th>
<th>Genotype</th>
<th>Transgenes</th>
<th>Method of transformation</th>
<th>Transformation efficiency/studies</th>
<th>References</th>
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<tr>
<td>Protoplasts</td>
<td>---</td>
<td><em>cat</em></td>
<td>Electroporation</td>
<td>Efficient gene expression</td>
<td>Ou-Lee <em>et al.</em>, 1986</td>
</tr>
<tr>
<td>Cell Suspensions/ Protoplasts</td>
<td>---</td>
<td><em>npt II</em></td>
<td>Electroporation</td>
<td>Stable transformation</td>
<td>Battraw and Hall, 1991</td>
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<td>Cell suspension culture</td>
<td>---</td>
<td><em>npt II, hpt, udi A</em></td>
<td>PDS-1000/ He (Bio-Rad)</td>
<td>Stable transformation</td>
<td>Hagio <em>et al.</em>, 1991</td>
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<tr>
<td>Immature embryos</td>
<td>---</td>
<td><em>bar, udi A</em></td>
<td>PDS-1000/ He (Bio-Rad)</td>
<td>Plants regeneration at low frequency</td>
<td>Casas <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Immature embryo/ inflorescence callus</td>
<td>---</td>
<td><em>bar, udi A &amp; luc</em></td>
<td>PDS-1000/ He</td>
<td>Plants regeneration at low frequency</td>
<td>Konono wicz <em>et al.</em>, 1995</td>
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<tr>
<td>Immature embryo/inflorescence callus</td>
<td>---</td>
<td><em>bar</em></td>
<td>Particle inflow gun</td>
<td>Single plant reported</td>
<td>Rathus <em>et al.</em>, 1996</td>
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<td>Immature inflorescence</td>
<td>P898012</td>
<td><em>gus</em> and <em>bar</em> genes</td>
<td>Microprojectile bombardment</td>
<td>0.286%</td>
<td>Casas <em>et al.</em>, 1997</td>
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<tr>
<td>Immature embryos</td>
<td>---</td>
<td><em>bar/Chitinase I</em></td>
<td>PDS-1000/ He</td>
<td>----</td>
<td>Zhu <em>et al.</em>, 1998</td>
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<tr>
<td>Immature embryo</td>
<td>---</td>
<td><em>bar</em> gene using CaMV 35S/act 1 as promoter</td>
<td>Particle inflow gun</td>
<td>Used Casein hydrolysate to enhance regeneration frequency</td>
<td>Rathus and Godwin, 2000</td>
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<tr>
<td>Immature embryos</td>
<td>P898012; PH1391 (Commercial line)</td>
<td><em>bar</em> gene for herbicide resistance</td>
<td>Agrobacterium mediated</td>
<td>2.1%</td>
<td>Zhao <em>et al.</em>, 2000</td>
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<tr>
<td>Immature embryos callus</td>
<td>---</td>
<td><em>uid A</em></td>
<td>Particle bombardment</td>
<td>Promoter studies</td>
<td>Hill-Ambroz and Week 2001</td>
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</tbody>
</table>

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

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<table>
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<tr>
<th>Process Type</th>
<th>Tissue/Plant Part</th>
<th>Reporter Gene(s)</th>
<th>Promoters Used</th>
<th>Transfection Method</th>
<th>Transformation Efficiency</th>
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<td>Immature embryo callus</td>
<td>---</td>
<td>uid A, bar</td>
<td>act I and ubi 1 as promoters</td>
<td>Particle bombardment</td>
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<td>Immature zygotic embryos; 7-10 day old embryogenic calli; leaves</td>
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<td>GFP and glucuronidase (gus) as reporter genes</td>
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<td>Reported gfp to be superior to gus</td>
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<td>Immature and mature embryos</td>
<td>The accession no. ‘214856’ from Ethiopia</td>
<td>dhdps raec I mutated gene; uidA as reporter gene</td>
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<td>Microprojectile bombardment</td>
<td>1.3%</td>
<td>Tadesse et al., 2003</td>
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<td>Shoot meristems</td>
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<td>bar/HVA I</td>
<td>CaMV 35 s promoter</td>
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<td>On Drought tolerance</td>
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<td>bar, cry I Ab &amp; cry IB</td>
<td></td>
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<td>nptII, dhdps-raec I</td>
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<td>---</td>
<td>Tadesse and Jacobs 2004</td>
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<td>hpt, npt, uid A</td>
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<td>Agrobacterium mediated</td>
<td>---</td>
<td>Carvalho et al., 2004</td>
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<td>---</td>
<td>gfp/bar/tlp/ri ce chitinase GII using ubi 1 promoter</td>
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<td>Agrobacterium mediated</td>
<td>---</td>
<td>Jeoung et al., 2004</td>
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<td>Shoot apices</td>
<td>BTx623</td>
<td>Cry 1 Ac gene, bar, Uid A gene using mpi CI as promoter</td>
<td></td>
<td>Co-bombardment particle inflow</td>
<td>1.5%</td>
<td>Girijashanker et al., 2005</td>
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<td>Immature embryo Callus</td>
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<td>gfp/tlp</td>
<td></td>
<td>Agrobacterium mediated</td>
<td>Marker free studies and southern blot for tlp reported</td>
<td>Gao et al., 2005</td>
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<td>Immature embryo callus</td>
<td>Tx430; C401 and a commercial hybrid</td>
<td>Dual marker plasmid containing manA and pmi gene</td>
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<td>Agrobacterium mediated</td>
<td>2.88% and 3.30%</td>
<td>Gao et al., 2005</td>
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<tr>
<td>Immature embryo type</td>
<td>Variety/Description</td>
<td>Selectable marker and Reporter gene</td>
<td>Transformation Frequency (%)</td>
<td>Reference</td>
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<td>Immature embryos</td>
<td>Pioneer 8580</td>
<td>phosphomannose isomerase as selectable marker and gfp as reporter gene</td>
<td>0.3-4.5%</td>
<td>Howe et al., 2006</td>
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<td>Immature embryos</td>
<td>Tx430; C2-97</td>
<td>Agrobacterium mediated npt II as selectable marker gene (hygromycin phosphotransferase gene)</td>
<td>5%</td>
<td>Nguyen et al., 2007</td>
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<td>Immature embryos derived calli</td>
<td>Red sorghum cultivar Sensako 85/1191 (Monsanto, South Africa)</td>
<td>npt II as selectable marker gene (hygromycin phosphotransferase gene)</td>
<td>5%</td>
<td>Nguyen et al., 2007</td>
<td></td>
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<tr>
<td>Pollen</td>
<td>---</td>
<td>npt II, uid A</td>
<td>PCR and Southern Blot reported 49.1% GFP-expressing calli and 8.3% stable transformation frequency</td>
<td>Wang et al., 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat treated Immature embryos</td>
<td>P898012</td>
<td>phosphomannose isomerase (PMI) as selectable marker; gfp as reporter gene</td>
<td>Agrobacterium mediated 0.4 and 0.7% Marker free transgenic sorghum studies</td>
<td>Gurel et al., 2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature embryos</td>
<td>P898012</td>
<td>High lysine protein gene HTL 2; bar gene as a plant selectable marker and the GUS-intron gene as a reporter</td>
<td>Agrobacterium mediated</td>
<td>Lu et al., 2009</td>
<td></td>
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<tr>
<td>Immature zygotic embryos</td>
<td>P898012</td>
<td>bar gene and man A for phosphomannose isomerase</td>
<td>Particle bombardment 0.77%</td>
<td>A. W. Grootboom et al., 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature embryo</td>
<td>Ramada (Sweet Sorghum)</td>
<td>hpt, Luc</td>
<td>Microprojectile bombardment 0.09%</td>
<td>Anshu Raghuvanshi and Robert G Birch, 2010</td>
<td></td>
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<tr>
<td>Immature zygotic embryos</td>
<td>Kat 412</td>
<td>Chitinase and Bar gene</td>
<td>Enhanced Linus Moses</td>
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</tbody>
</table>
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Table 3: Molecular approaches for crop improvement using various molecular markers

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

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., 1993).
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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 Comparative 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 sorghums. 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 (Viktor, 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
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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 Acroframium 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 dense 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 in turn 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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REFERENCES


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