**Improvement of Sorghum through Transgenic Technology**

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Sorghum is the fifth most important cereal crop in the world. It is largely grown on marginal soils with residual moisture where other major cereals cannot be grown due to inadequate water. Sorghum is a multipurpose crop and the species shows great diversity. For a large part of Asia and Africa, sorghum’s grain is used as food and its stalk as fodder and feed. In rest of the world, sorghum is considered as forage crop and also as stock for ethanol production.

Sorghum yield has been substantially increased through conventional breeding in India. However, resistance to abiotic stresses and biotic stresses such as shoot fly, stem borer, grain mold, and charcoal rot is limited due to inadequate genetic resources that can be readily used in crop improvement programs. Therefore, genetic engineering technology can assist the production of agronomically desirable crops that exhibit increased resistance to pests, pathogens, and environmental stress and enhancement of nutritional qualities.

Sorghum research has received less attention compared to other cereals for adoption of modern molecular tools, and very few laboratories in the world are addressing sorghum crop improvement programs through novel methods. Extensive research has been focused on other cereal crops, and a number of genes conferring agronomic advantages have been introduced through *Agrobacterium* and particle bombardment. In this article, we present the current status, progress, and prospects in transgenic sorghum technology and future approaches to increase its economic value, thereby providing monetary benefits to sorghum farmers.

**Sorghum Transformation**

The first report of the successful transformation of sorghum appeared as early as the 1990s. Yet, sorghum is considered to be the most recalcitrant crop for tissue culture and plant regeneration, thereby for genetic transformation. Recalcitrance in sorghum tissue culture is reportedly due to the release of phenolics, lack of regeneration in long term *in vitro* cultures, and a high degree of genotype dependence. The release of phenolics into the culturing medium can be overcome by frequent subculture and by the addition of polyvinyl pyrrolidone phosphate (PVPP) in the medium. However, transformation followed by regeneration remains extremely complicated in sorghum transgenic technology.

Genetic transformation of sorghum has picked up momentum in recent years, with a greater number of reports published in the last couple of years. Though different explant sources such as immature inflorescence, immature embryo, and shoot meristem are reported in sorghum transformation, successful recovery of transgenic plants through *Agrobacterium* mediated and particle bombardment are mainly achieved using immature embryos. In general, it takes 10 – 12 months for a highly-responding immature embryo to regenerate into a transgenic plant, despite much labor-intensive work.

Though both systems of transformation, i.e., *Agrobacterium*-mediated and particle bombardment, are successful in sorghum, the most effective method to date is *Agrobacterium* based transformation, with a high transformation efficiency that ranges from 2.1% – 4.5% (Howe et al., 2006; Gao et al., 2005; and Zhao et al., 2000). Zhao et al. (2000) reported the first production of transgenic sorghum plants by *Agrobacterium*-mediated transformation using immature embryos. They have tested a number of parameters that include optimization of media, concentration of bacterial culture, and duration of co-cultivation, for delivery of t-DNA in immature embryos.

Tadesse et al. (2003) optimized transformation conditions through the biolistic approach for production of transgenic sorghum plants. Transgenic sorghum plants were produced using immature zygotic embryos combined with optimized transformation conditions and a strong monocot gene promoter. Transformation efficiency was marginally higher (1.3%) than earlier reports of transgenic sorghum (0.08 – 1%) via microprojectile bombardment.

Girijashankar et al. (2005) reported successful recovery of transgenic sorghum plants by particle bombardment of shoot apices and production of transgenic plants, with a transformation frequency of 1.5%. Though production of transgenic plants using shoot apices or meristems reduces the time involved in regenerating transgenic sorghum plants, the associated limiting factors for their suitability for efficient production of transgenics are the i) additional skills required for the isolation of meristems; ii) frequent need for subculture/clippings; and iii) possibility of production of transgenic chimeras.

**Crop improvement traits**

Sorghum is chiefly grown in low input conditions; therefore, development of host plant resistance to biotic and abiotic stresses is a viable option. Demand for sorghum as a health food is gaining importance, and thus, incorporation of certain value added traits is advantageous to the food industry.

Worldwide, sorghum producers face a major threat to their crops from insect pests, and the most destructive pests are the lepidopteran stem borer (*Chilo partellus*) and the dipterans, midge (*Stenodiplosis sorghicola*) and shoot fly (*Atherigona soccata*). Building resistance through conventional breeding is limited due to a lack of reliable resistance sources. Insecticidal crystal proteins (CRY) from *Bacillus thuringiensis* are very effective against the lepidopterans and dipterans. Bt and other genes with insecticidal activities are being evaluated for eventual use in transforming crops and reducing losses due to these pests.
Girijashankar et al. (2005) produced transgenic sorghum plants carrying a synthetic gene, Bt cry1Ac, under the control of a wound inducible promoter from a maize protease inhibitor gene (mpi). They reported low levels of Bt protein of 1–8 ng per gram of fresh leaf tissue. A moderate level of tolerance was reported, which in turn conferred partial protection against neonate larvae of the spotted stem borer (Chilo partellus).

Padmaja produced transgenic plants carrying a synthetic gene Bt cry1B under the control of the constitutive promoter maize ubiquitin (ubi) in the parental lines of Indian hybrids via particle bombardment. Some of the events are promising in insect bioassays with 80% larval mortality compared to non-transformed control plants (Padmaja, personal communication).

The agronomically important gene chi II, encoding rice chitinase under the constitutive CaMV 35S promoter, has been transferred to sorghum for resistance to stalk rot (Fusarium thapsinum) by Zhu et al. (1998) and Krishnaveni et al. (2001).

Trials are also underway to engineer sorghum to withstand abiotic stress conditions, such as drought and salinity. Efforts are in progress to transfer genes milD, p5CSf129A, and codA to Indian sorghum genotypes for biosynthesis of osmoprotectants. Expression of these genes leads to accumulation of osmolytes, resulting in tolerance to various abiotic stresses (Maheshwari et al., personal communication). Overexpression of the gene for mannitol-1-phosphate dehydrogenase (milD) for biosynthesis of mannitol enhances tolerance to water deficit stress, primarily through an osmotic adjustment that improves growth of transgenic plants under water stress and salinity. The p5CSf129A gene codes for pyrroline-5-carboxylate synthase, which catalyses the first two steps of proline biosynthesis in plants. codA codes for choline oxidase, which converts choline into glycine betaine.

Sorghum grain is loaded with starch and is relatively poor in protein and lipid. Tadesse and Jacob (2003) introduced the dhdps-raecI mutated gene, which encodes an insensitive form of dihydrodipicolinate synthase, the key regulatory enzyme of the lysine pathway. Overexpression of the gene produces sorghum lines with elevated lysine content. Enrichment of the essential amino acid lysine in sorghum grain improves nutritional quality. Efforts are also underway to transfer the high molecular weight (HMW) wheat glutenin gene 1Ax1 into sorghum to alter dough quality to meet demands from the bakery industry (SV Rao, personal communication).

Biosafety concerns

There are no transgenic sorghum crops under commercial cultivation to date. The most important issue related to biosafety concerns in sorghum is pollen-mediated gene flow to the wild species Sorghum halepense (Johnsongrass), a wild weedy relative, reported to occur naturally at frequencies of 2.5% at a distance of 13m (Schmidt and Bothma, 2006). The concern is that transfer of the herbicide tolerance gene to S. halepense through gene flow would make control of the weed unattainable. Godwin (2005) reported that hybridization of S. halepense (2n=40) and cultivated sorghum, S. bicolor (2n=20), would produce unviable triploids. Transgenic technology in sorghum is at a juvenile stage. Recently Gao et al. (2005) established transgenic technology using the positive selectable marker gene pmi (phosphate mannose isomerase), which is biosafe and found widely in other crops.

Future prospects

Despite the use of other monocot promoters such as rice actin (act-1) and maize ubiquitin (ubi1), use of native promoters in sorghum may be explored if it helps to increase the levels of transgene expression. In the eukaryotic genome, DNA elements called scaffold/matrix attachment regions (MARs) are primarily involved in structural and functional organization. They are thought to influence gene expression, and evidence from other transgenic crops reveals that these sequences, when flanking the transgene, result in enhanced expression of the integrated gene(s). Research in making potentially well-defined synthetic MARs and improving stable transgene expression in sorghum are areas of immediate attention. Construction of a detailed genetic map of sorghum is underway, and information from these efforts will facilitate the identification of promoters that enhance transgene expression. The agronomic potential of transgenic sorghum to improve nutritional value, increase productivity, and withstand biotic and abiotic stresses will depend on the development of effective transformation technology and improved selectable marker genes.

References
