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Sorghum Transformation: Overview and Utility

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Abstract

Over the past decade genomics resources available for sorghum have rapidly expanded (Paterson Int J Plant Genomics 2008:6, 2008), these resources, coupled with the recent completion of the genome sequence which is relatively small in size (730 Mb) (Paterson et al. Nature 457:551–556, 2009) makes sorghum a rather attractive species to study. Moreover, the USDA germplasm system maintains 42,614 accessions, of which more than 800 exotic landraces have been converted to day length-insensitive lines to facilitate their use in breeding programs. In addition, a set of EMS mutation stocks developed by the USDA

Plant Stress and Germplasm Development Unit in Lubbock, TX (Xin et al. Bioenerg Res 2:10–16, 2009) will be a valuable resource for functional genomics studies in sorghum. However, in order to be a robust system for study a suite of functional genomics tools are necessary to complement these other resources to aid in down-stream hypothesis testing. A key functional genomics tool is the ability to modulate gene expression through the introduction of transgenic genetic elements. This is exemplified by recent work (Cook et al. Plant Cell 22:867–887, 2010) in which RNAi experiments were employed to specifically reduced expression of two alkylresorcinol synthases to demonstrate their role in the synthesis of the allelopathic molecule sorgoleone. In addition to its value as a functional genomics tool, plant transformation offers a route to broaden access to novel input and output traits for sorghum breeding programs.

Keywords: *Agrobacterium tumefaciens*, Transformation, *npt II*, Biotechnology, Sorghum Genetic engineering

1. Sorghum Transformation

In general plant transformation can be partitioned into two components: competence of a cell for culture regeneration into a whole plant and receptiveness of that same cell for foreign DNA integration. In sorghum, like most monocotyledonous plants, in vitro culture regimes are primarily somatic embryogenesis based systems (Elkonin and Pakhomova 2000; Jogeswar et al. 2007; Kaeppler and Pedersen 1996; Pola et al. 2008; Pola and Mani 2006; Sato et al. 2004*a*). As per the second component of plant transformation, integration of genetic elements, sorghum has been successfully transformed using both direct DNA delivery methods (Battraw and Hall 1991) and *Agrobacterium*-mediated transformation protocols (Cai et al. 2002; Gao et al. 2005*a*, *b*; Gurel et al. 2009; Howe et al. 2006; Nguyen et al. 2007; Zhao et al. 2000). While both DNA delivery systems are proven technologies for recovery of stable sorghum transformants, more laboratories are moving towards implementing the latter due to the tendency of *Agrobacterium*-mediated transformants to carry lower copy number insertions and/or have a higher frequency of coexpression of the nonselected transgenic cassette (Dai et al. 2001; Gao et al. 2008; Zhao et al. 1998).

While multiple explants have been evaluated as the starting material for sorghum transformation, clearly the primary explant reported on is immature embryos. One of the factors that have hampered transformation efficiencies of sorghum with the immature embryo explant is the rapid production of phenolic compounds. Phenolics are produced during the in vitro culturing of sorghum immature embryos, but the production of these secondary metabolites is enhanced upon inoculation with *A. tumefaciens*. To alleviate the negative effects of phenolics on sorghum transformation media supplements such as polyvinylpolypyrrolidone (PVPP) (Cai et al. 1987), and elevation of potassium phosphate levels (Elkonin and Pakhomova 2000; Sato et al. 2004*a*), or the exposure of explants to reduced temperature (Nguyen et al. 2007) have been shown to be able to reduce, but not totally eliminate the negative impact of these compounds. Triggering of the plant's defense response upon challenge with *A. tumefaciens* may lead not

only to the production of secondary metabolites, but also to cell death, which can further hamper the efficiency of recovery of transgenic plants. For example in banana the triggering of apoptosis by *A. tumefaciens* can be effectively countered by the expression of antiapoptotic genes (Khanna et al. 2007). While such a strategy has not been evaluated in sorghum, a heat shock pretreatment, which was previously reported to counter apoptosis in banana embryogenic callus, leading to improved transformation efficiency (Khanna et al. 2004), has recently been shown to be a translatable technique using sorghum immature embryos (Gurel et al. 2009).

Key to any transformation system is the ability to rapidly, and efficiently distinguish transgenic differentiating cells from nontransgenic cell lineages. Two means typically used to differentiate transgenic from nontransgenic cell lineages are the use of visual or selectable marker genes. In sorghum the visual marker genes green fluorescent protein (gfp) and β-glucuronidase (GUS) are each effective in monitoring for transgenic cells (Jeoung et al. 2002). Using the former visual marker Gao et al. (2005*a*) reported a 3.0% transformation efficiency as means to monitor for transgenic differentiating cell lineages from immature embryos of sorghum.

Selectable marker genes used to provide a competitive edge in culture for plant transformation systems typically rely upon providing resistance to antibiotics, such as hygromycin (Gritz and Davies 1983), and the aminoglycoside kanamycin, or various derivatives thereof (Fraley et al. 1983) or tolerance towards herbicidal agents glyphosate (Barry et al. 1992) and glufosinate (Thompson et al. 1987). In addition the positive selectable marker gene phosphomannose isomerase (PMI) (Joersbo and Okkels 1996) has been shown to be a rather robust selection system for the identification of transgenic plants (Negrotto et al. 2000), including sorghum (Gao et al. 2005*b*).

1.1 *Outline of an Agrobacterium-Mediated Transformation of Sorghum Using npt II as a Selectable Marker Gene*

As indicated above there have been multiple reports of successful transformation of sorghum following the communicated success in 1993 (Casas et al. 1993). Outlined below is the system reported on by Howe et al. (2006) that utilizes *npt II* as the selectable marker gene, coupled with G418 a the selection agent. While the overall transformation efficiency with this system is relatively low, typically ranging from 1 to 3%, the system is consistent, and importantly teachable with minimal training.

This sorghum transformation system relies upon immature embryos as the starting material. One of the disadvantages of using this explant is the need for continual plantings of stock plants to ensure a constant supply of immature embryos, adding labor and cost to the system. Nonetheless, the stock plants used to supply immature embryos are maintained under greenhouse conditions. Heads are harvested when 70% of the head have embryos ranging in size from 1.2 to 2.2 mm in length. Each head is then excised from the plant and placed in a 1,000 ml graduated cylinder filled with 500 ml of 50% commercial bleach plus

250 ml of Tween 20. The cylinder containing the sorghum head submerged in the bleach solution is mixed with a stir bar for 20 min. Following the 20 min surface sterilization with bleach the heads are washed three times with sterile water and allowed to air dry in laminar flow hood. Seeds are isolated and subjected to a secondary sterilization process consisting of a 1 min soak with agitation in 70% ethanol, followed by a single wash with sterile water, proceeded by a soak with agitation in 10% bleach solution, with a subsequent triple wash in sterile water.

Immature embryos are isolated from 50 sterilized seeds and placed in a 35 × 10 mm Petri plate containing 1 ml of coculture medium supplemented with 300 μM of acetosyringone. The coculture medium is composed of 0.5× MS major and minor salts (Murashige and Skoog 1962), 0.5 mg/l each of nicotinic acid and pyridoxine HCl, plus 1 mg/l each of thiamine HCl and casamino acids. The carbohydrate sources are 2% sucrose and 1% glucose and growth regulator, 2,4-d, at 2 mg/l. The medium is buffered with 3 mM MES (pH 5.2). The medium is filter sterilized, with no components being autoclaved.

Once 50 immature embryos have been isolated the coculture medium is removed and replaced with 1 ml of *A. tumefaciens* inoculum. The inoculum is an *A. tumefaciens* strain NTL_4/pTiPKPSF2 (Palanichelvam et al. 2000), suspension in cocultivation medium (OD_{660} 0.3–0.5). Inoculation time is 5 min. Following the cocultivation step the explants are placed scutellum side up on 100 × 20 mm Petri plates containing four sterile Whatman™ filter papers saturated with 4.2 ml of cocultivation medium. The plates are incubated for 2 days at 24°C in the dark.

Following the cocultivation period the explants are cultured on delay medium which is composed of Elkonin's major salts (Elkonin and Pakhomova 2000), MS minor salts and vitamin mix, 2 g/l proline, and 1 g/l asparagine. The carbohydrate source is 3% sucrose, the medium is buffered with 3 mM MES (pH 5.7) and solidified with 2% phytagel. To counter select against A. tumefcaiens the medium is supplemented with 100 mg/l carbenicillin. The growth regulator 2,4-d is used at a level of 1.5 mg/l. Cultures are incubated at 28°C in the dark for 3 days.

The selection phase is immediately implemented following the delay period. A total of 20 embryos are placed on to 100 × 20 mm Petri plates containing the delay medium supplemented with 20 mg/l G418. The tissue is transferred to fresh selection medium every 2–3 weeks. As coleoptiles develop they are systematically removed from the explants. As the embryogenic callus begins to form about the explants the tissue is broken up into 1–3 mm pieces, with care being taken to ensure tracking of tissue from the original explant, given the fact that most transformants derived from the same initial immature embryo tend to be clones, and hence the best way to track transformation efficiencies. The callus tissue remains in the selection phase for a period of 6–9 weeks.

Following the selection phase proliferating embryogenic tissue is transferred to regeneration medium composed of MS major/minor salts and vitamins, supplemented with 0.5 mg/l kinetin and 1.0 mg/l IAA. The medium is solidified with 2% phytagel, carbohydrate level, 3% sucrose, and buffered with 3 mM MES (pH 5.7). The selection pressure is reduced to 10 mg/l G418, and the carbenicillin level remains at 100 mg/l. The cultures are placed under a 16/8 light regime at

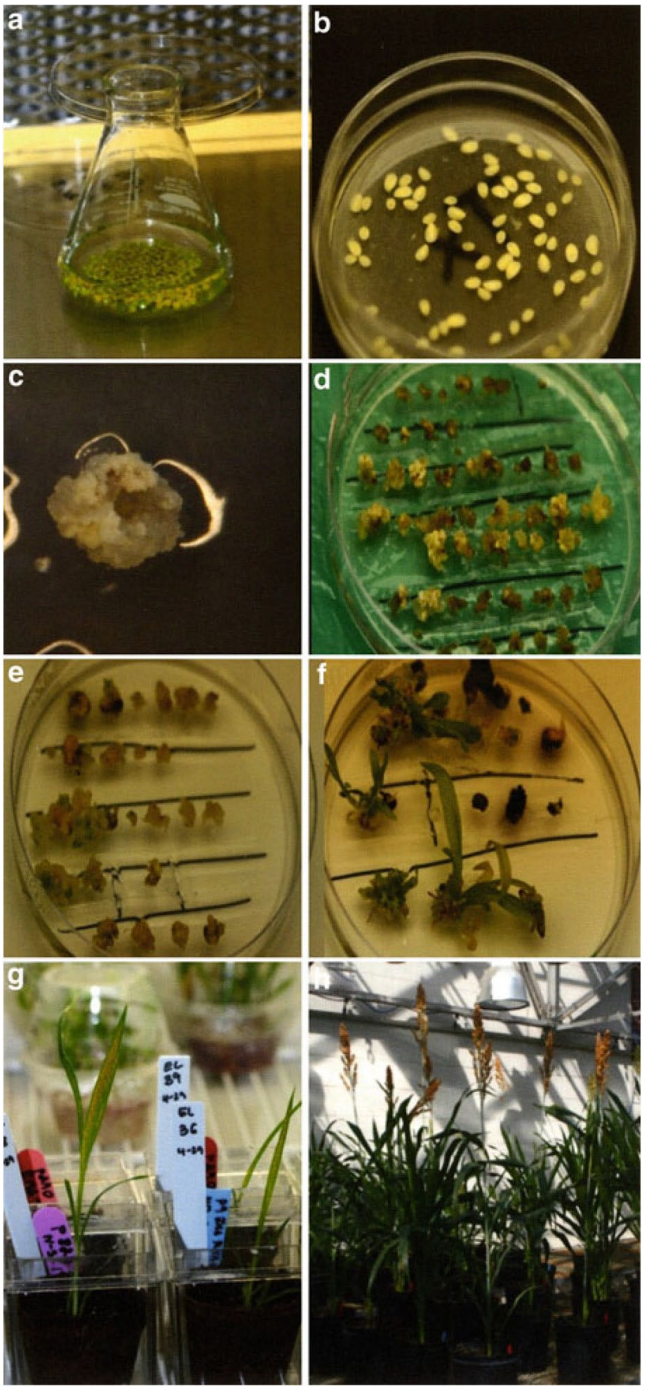

Figure 1. Overview of sorghum transformation steps. (a) Sterilization step of immature seeds. (b) Inoculation step of immature embryos. (c) Somatic embryogeneic tissue. (d) Partitioning of somatic embryogenic tissue to ensure lineage tracking. (e, f) Regeneration steps. (g) Acclimation of plants. (h) Transgenic sorghum in greenhouse.

24°C. Typically after 4 weeks on regeneration, with one subculture at the 2-week period, shoots with well established roots will form, that are ready to be acclimated to soil (Figure 1).

Once acclimated the primary transformants are confirmed using a commercial ELISA kit to monitor *npt II* expression (Agdia Corp.). Primary transformants are screened to identify a minimum of two lead events for down-stream characterizations. A lead event is selected based on simple integration pattern of the transgenic element(s), and expression of the target phenotype(s) of interest.

2. Considerations in Designing Binary Vectors for Sorghum Transformation

An attribute of *Agrobacterium*-mediated transformation is that T-DNAs can be integrated, albeit relatively infrequently, at unlinked positions. This ability of *A. tumefaciens* can be exploited to derived marker-free transgenic events through the simultaneous delivery of two T-DNAs, where one of the T-DNA elements carries the marker gene, and the other carries gene(s) of interest. If integrated at unlinked positions, the T-DNAs, will segregate in the progeny. This strategy has been successfully used to derived maker-free transgenic plants in a number of systems (Daley et al. 1998; Jacob and Veluthambi 2002; Komari et al. 1996; Sato et al. 2004*b*; Xing et al. 2000), including sorghum (Zhao et al. 2003). The integration of unlinked T-DNA alleles in sorghum is exemplified in Figure 2. Transgenic sorghum events were generated that harbored a transgenic cassette with the cyanamide hydratase (cah) gene (Maier-Greiner et al. 1991), under control of the sugarcane polyubiquitin promoter ubi4 (Wei et al. 2003), housed within a single T-DNA binary vector designated pPTN181 (not shown). A Southern blot analysis is performed using a restriction enzyme wherein one recognition site resides within the T-DNA element, hence each hybridization signal will reflect a single integration locus, on a subset of primary transformants derived from pPTN181 as shown in Figure 2a. As can be seen the event 168 carries one locus, while events 165 and 166 harbor two loci, and event 164 contains three loci. Monitoring segregation of the transgenic alleles in progeny derived from these events revealed a 15:1 pattern for events 165 and 166, and a 3:1 pattern for events 164 and 168 (data not shown). Southern analysis on a subset of the derived progeny is in agreement with the observed segregation patterns (Figure 2b, c). It can be seen in the T_1 individuals derived from events 165 and 166 that some individuals display the genotype of the parent, while others only carry one of the transgenic alleles. On the other hand all T_1 derived from event 164 had the same genotype as the parent, hence all alleles appear to be linked. However the single locus event, 168, segregated as expected, with T_1 individuals genotyped the same as the parent.

When implementing the tool of plant transformation for targeted output and input traits in sorghum such as improvement in grain quality or stress tolerance, respective, it is critical to have promoter elements with the desired specificity so to limit the probability of negatively impacting agronomic performance that may arise if the phenotype is misexpressed in nontarget tissues. To this end it is prudent to verify promoter specificity if using a promoter known to be tissue specific

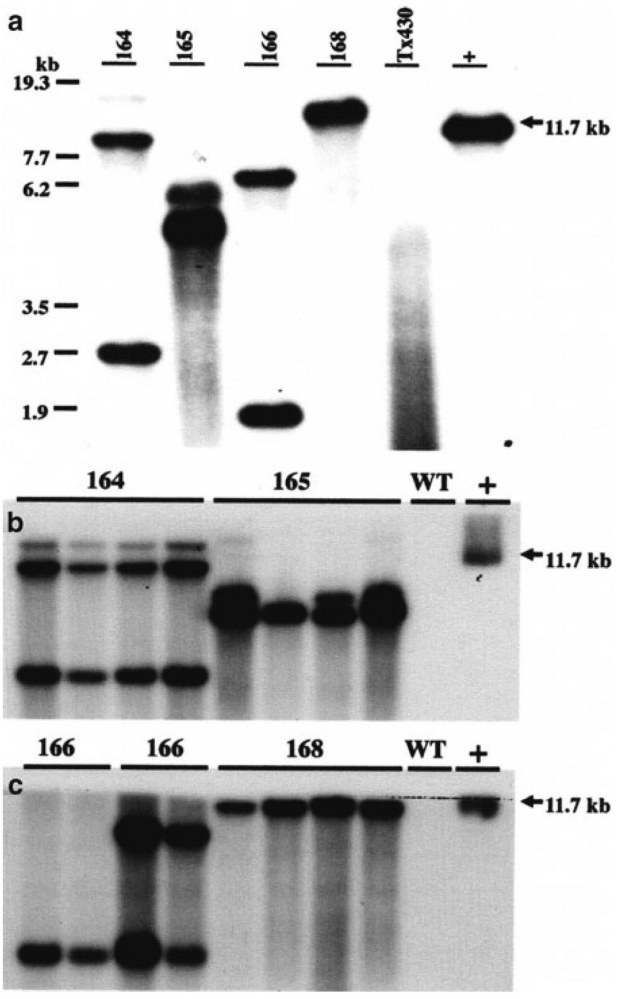

Figure 2. Southern blot analysis of transgenic sorghum events carrying cyanamide hydratase (cah) gene. (a) Primary transformation events designated 164, 165, 166, and 168 probed with cah ORF. + lane indicates 50 pg linear binary vector pPTN181. Tx430 lane is 10 µg of wild type DNA. (b) Southern blot analysis of T_1 progeny derived from events 164 and 165, highlighting segregating transgenic alleles in event 165, and linked alleles in 164. WT lane indicates 10 µg genomic DNA from Tx430. + lane is 50 pg of linear binary vector pPTN181. (c) Southern blot analysis of T_1 progeny derived from events 166 and 168, highlighting segregating transgenic alleles in event 166, and linked locus in 168. WT lane indicates 10 µg genomic DNA from Tx430. + lane is 50 pg of linear binary vector pPTN181.

in other species, before assembling cassettes for use in sorghum. For example interest in modifying seed components of sorghum may require specific expression in the embryo. A logical candidate promoter for desired embryo-specific expression would be the maize globulin-1 promoter (Belanger and Kriz 1991). To evaluate whether the glob-1 promoter specificity would translate to sorghum a GUSPlus™ (www.cambia.org) cassette under control of the glob-1 promoter was assembled and introduced into sorghum. As a constitutive control transgenic sorghum carrying a GUSPlus™ cassette under control of the maize polyubiquitin promoter (Christensen et al. 1992) was used for comparison. Tissue samples were assayed over development in T_1 or T_2 individuals, looking at GUS expression within root, leaf, stem, glume, scutellum, and embryos. As can be seen in Figure 3, embryo-specificity of the maize glob-1 promoter does effectively translate to sorghum. While this result is not surprising, these are data that need to be gathered to fully exploit sorghum transformation as a translational genomics tool.

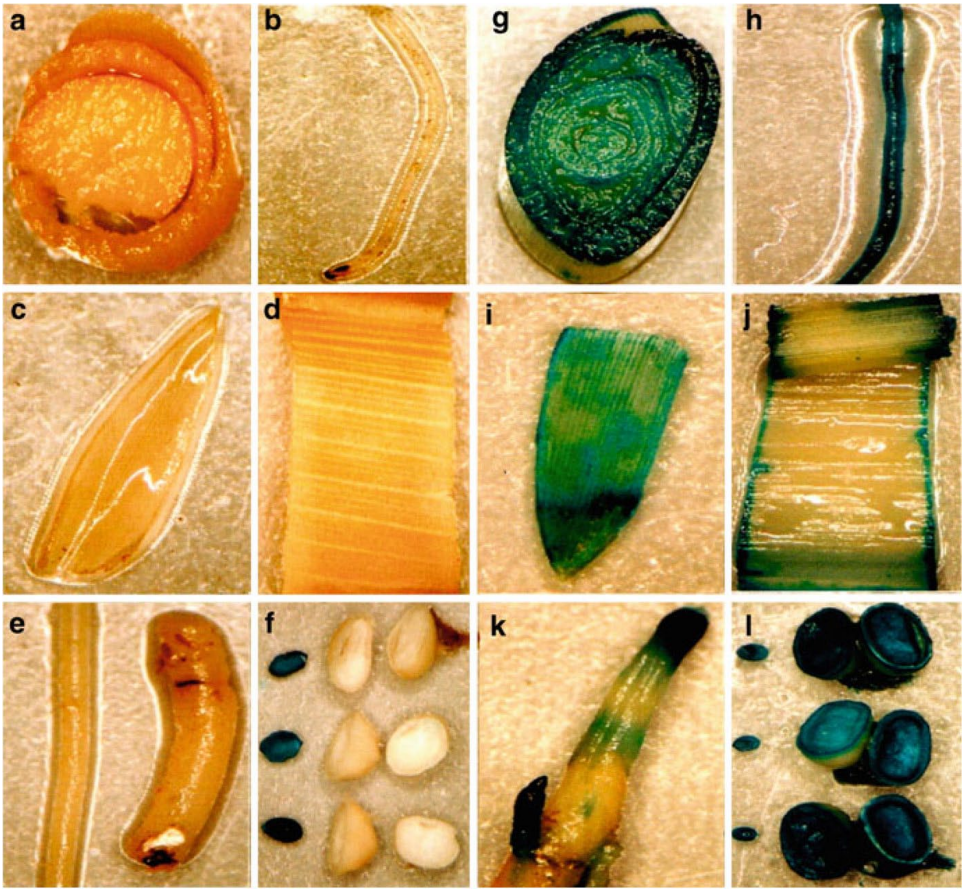

Figure 3. GUS expression profile observed in transgenic sorghum. Panels (a–f): Transgenic sorghum event carrying the glob-1-GUS cassette showing embryo specific expression. (a) Stem section, (b) root section, (c) glume, (d) leaf, (e) root, and (f) seed, endosperm and embryo (blue). Panels (g–l): Transgenic sorghum event carrying the ubiquitin-1-GUS cassette showing constitutive GUS expression. (g) Stem section, (h) root section, (i) glume, (j) leaf, (k) root, and (l) seed, endosperm and embryo.

3. Target Input Traits for Sorghum Through Transformation

A critical trait for any breeding program is yield. Addressing yield directly through transgenic approaches is a rather large challenge. A more practical and obtainable goal in the short term is protection of yield through control of biotic and abiotic stresses. In sorghum, like most crops, key stresses that compromise yield will vary across regions. Sorghum production can be severely impacted by a number of insect pests. Not only can insects impact production directly, but in some cases they can also provide an entry for secondary pathogen attack at the site of insect feeding. The success of the *Bt* technology in maize (Armstrong et al. 1995; Barry et al. 2000), and cotton (Cattaneo et al. 2006), is a strong rationale for

the evaluation of this technology in sorghum as a means to combat specific target insect pests. Importantly, in addition to *Bt*'s direct impact in impeding insect pest feeding damage, a secondary effect observed with the use of this technology is a significant reduction in accumulation of various mycotoxins in plant tissues (Abbas et al. 2008; Bakan et al. 2002; Hammond et al. 2004). This secondary attribute of the *Bt* technology may serve as a valuable mechanism to limit quality issues of sorghum related to contamination of these toxins that may occur under certain conditions and fungal infestation levels (Ghali et al. 2009; Reddy and Raghavender 2008; Reddy et al. 2010). However, like all disease resistance traits, the *Bt* technology needs to be used in conjunction with proper integrated pest management practices to maximize its durability over time (Kumar and Pandey 2008; Sharma and Ortiz 2000).

A number of viral agents have been shown to be capable of replication in sorghum (Jensen and Giorda 2002), including members of the potyvirus family including sugarcane mosaic virus, maize dwarf mosaic virus, and sorghum mosaic virus. Limited resistance towards these viral agents has been identified within sorghum germplasm, although some reports have been communicated (Henzell et al. 1982). The seminal work which demonstrated introduction of viral coat protein genes in transgenic plants to confer virus resistance (Abel et al. 1986; Nelson et al. 1987; Stark and Beachy 1989), has opened the door for the translation of this technology to other plant systems, implementing various genetic constructs that target silencing of critical gene products required for the replication of the virus of interest (Beachy et al. 2003; Prins 2003), including known pathogens of sorghum (Gilbert et al. 2005). Hence, such strategies offer great potential for the introduction of durable virus resistance for sorghum.

Striga, commonly referred to as witch weed, contains two species, *S. hermonthica* and *S. asiatica*, that are parasitic on sorghum and other cereals (Aly 2007). Parasitic plant species infest nearly 50 million hectares crop plants on an annual basis, and great strides have been made in developing resistance in sorghum through conventional breeding approaches (Ejeta 2007). More recently Tuinstra et al. (2009) have communicated a herbicide seed treatment strategy that exploits the introgression of acetolactate synthase (ALS) herbicide resistance from shattercane into elite sorghum genotypes (Hennigh et al. 2010). Implementing this seed-coating approach significantly reduced *Striga* emergence under both greenhouse and field tests (Tuinstra et al. 2009). While this is a very promising tool to combat this devastating parasite, given that ALS inhibiting herbicides are typically classified as high risk for development of resistance, the durability of such a strategy may be limited without proper management. Hence, other approaches are needed to ensure long-term control towards Stiga. To this end, there has been a report looking at targeting critical genes in parasitic plant's life cycle by expression of hair-pin constructs in the host plant which resulted in an enhanced tolerance phenotype in the *Orobanche aegyptiaca*/tomato host parasite interaction (Aly et al. 2009). However, this approach was not successfully translated as a means to control to the *Striga*/maize parasite interaction (Yoder and Scholes 2010). Clearly additional research is required to further our understanding of the underlying biology involved during the early stages of parasitism by these plants. More efforts

around the assembly of -omics databases that carry this information (Torres et al. 2005) are needed in order to facilitate the development of alternative control strategies towards *Striga*, that may serve as a complement to the herbicide seed coating approach (Tuinstra et al. 2009).

Addressing a plant's response to stresses that are governed in a multigenic fashion is more challenging than single gene traits. In order to investigate multigenic abiotic stress response traits such as drought and heat, researchers are evaluating a coordinated expression of a suite of genes triggered by exposure to the targeted stress by the introduction of a single transcription factor (Karaba et al. 2007; Nelson et al. 2007; Suzuki et al. 2005). Theses transcription-factor based technologies hold great promise as a means to reduce multigenic expressed phenotypes to a single transgene fashion (Century et al. 2008), however, the transcription factor based strategy undou*bt*edly will require tight regulation, necessitating the need for tissue-specific and/or inducible promoter systems.

With respect to adaptation to low nitrogen environments, Yanagisawa et al. (2004) demonstrated that expression of the maize *Dof1* transcription factor improved nitrogen assimilation in transgenic plants. However, it is feasible to directly perturb nitrogen flux in plants. Nitrogen assimilation and metabolism in plants occurs through coordinated action of a variety of enzymes acting upon a variety of substrates. Two key enzymes involved in nitrogen metabolism in plants are glutamine synthetase (GS) and glutamate synthase (GOGAT). Previous studies have shown that enhancing GS or GOGAT activities can impact nitrogen metabolism in plant species (Cai et al. 2009; Good et al. 2004). Enhancing activity of another enzyme that impacts nitrogen flux in plants, alanine aminotransferease (Ala-AT), that catalyzes the production of alanine and 2-oxoglutarate from pyruvate and glutamate, has been shown to augment nitrogen use efficiency in both rape seed and rice (Good et al. 2007; Shrawat et al. 2008).

A caveat to these studies communicating enhancing nitrogen use efficiency through transgenic approaches is that most reports used data sets gathered from greenhouse or growth chamber studies, with minimal information on the impact of the respective transgenes on yield under field conditions (Brauer and Shelp 2010). Moreover, no data sets have been communicated to date on the impact of gene stacking strategies on nitrogen use efficiencies with these selected genes.

4. Target Output Traits for Sorghum Through Transformation

Digestibility of sorghum limits protein availability, and ultimately impacts the nutritional quality of the grain (Duodu et al. 2003). The major proteins, prolamins, found in sorghum reside in the endosperm. The prolamin storage proteins found in sorghum and maize are designated kafirins and zeins, respectively. The prolamins are assembled into protein bodies, with a very defined pattern, where the α class reside in the core along with the δ class, albeit to a lower extent, while the β and γ classes decorate the periphery of the protein body (Wu and Messing 2010). A number of parameters can influence

digestibility of sorghum protein, including structure and shape of the protein body (Duodu et al. 2003). Reduction of the zein proteins found in the maize mutants *opaque-2* (Hartings et al. 1989) and *floury-2* (Coleman et al. 1995) leads to a concomitant increase in lysine and tryptophan due to a compensation mechanism in seeds resulting in an increase in nonzein proteins (Coleman and Larkins 1999). Deliberate reduction in the 19 kDa α-zeins in maize manifests the opaque kernel phenotype, and enhances levels of lysine and tryptophan in the grain (Huang et al. 2004). Similarly, reduction in the level of both the β- and γ-zeins resulted in drastic changes protein bodies, and triggered the opaque kernel phenotype (Wu and Messing 2010). Hence, modulation of the prolamins is a target that could be pursued in sorghum as a means to simultaneously address digestibility, and nutritional quality.

Oria et al. (2000) described a highly digestible, enhanced lysine sorghum mutant. The protein bodies observed within this mutant are highly folded, with a redistribution of the γ-kafirin about the body. These factors lead to increased exposure of the core α-kafirins, which translates to the increased digestibility phenotype (Duodu et al. 2003).

Like the maize *floury-2* and *opaque-2* mutants, the highly digestible, enhanced lysine mutant of sorghum has value in both food and feed applications. However, translation of these traits to application has yet to be realized, undoubtedly due to the tendency of these altered prolamin grains to have reduced agronomic properties, and post harvest issues (Huang et al. 2004). However, these drawbacks may not be insurmountable. Breeding efforts are making progress in addressing the issues blocking deployment of the high digestible, enhanced lysine mutant of sorghum (Tesso et al. 2006, 2008*a*). Through better understanding of the underlying biology governing protein deposition in these mutants and the influence of the various genetic modifiers, will lead to improved biotechnology approaches, coupled with better breeding strategies, to modulate the seed storage proteins, without negatively altering the endosperm characteristics. Hence, in the end, the successful deployment of a high quality grain sorghum will ultimately require an interdisciplinary approach that brings together the expertise of plant breeding, biotechnology, molecular biology/genetics and food science.

5. Potential of Outcrossing

One of the concerns raised about release of transgenic sorghum is the potential for outcrossing to its weedy relatives, johnsongrass and shattercane, which has been hypothesized to potentially lead to altered balance in the ecosystem, changes in the plant community structure, and persistence of weeds in agricultural lands (Tesso et al. 2008*b*).

A number of parameters must be met for a successful sorghum outcross event to occur. First, the crop and weed species must be in close proximity, and flowering times synchronized. Hybrids derived from outcrosses between grain sorghum and shattercane (*S. bicolor* subsp. *drummondii* Nees ex Steud de Wet &

Harlan) do not appear to be compromised in fitness (Sahoo et al. 2010). This lack of fitness drag in sorghum × shattercane hybrids has a benefit when introgressing desirable alleles from the shattercane into the cultivated genotypes, for example ALS resistance (Hennigh et al. 2010). However, this attribute that benefits conventional breeding strategies used to broaden diversity of cultivated sorghum, negatively impacts the use of transformation as a tool for introduction of novel traits into the crop, given there will undoubtedly be a call for more extensive regulatory testing addressing the potential ecological impact of a given transgenic sorghum event, which in turn will lead to higher costs and delay in release, with getting a transgenic sorghum event on the market.

One approach that may limit the concern of transgenic sorghum impacting the ecosystem above what is already occurring with production of conventional cultivars is the use of a male sterility system that may effectively limit pollen flow of transgenic sorghum under field conditions (Pedersen et al. 2003). However, such containment systems in many cases may not be required. The current regulatory system has a "one size fit all approach," in that regardless of the trait developed through transgenic approaches, a series of laborious and costly studies must be conducted. While it is very reasonable to assume that deployment of a transgenic sorghum event will eventually outcross to a wild relative, this must not be the deciding factor to block production. Rather than a one size fits all model, perhaps a more thoughtful, scientific regulatory process, in which decisions are made on a case-by-case model is more appropriate. Recently, Hokanson et al. (2010) have communicated a straightforward and scientific-fact based risk assessment process that hopefully will open the door for more dialog in this area, and ultimately will allow for advances in transgenic technologies to enter the marketplace expeditiously in a safe and effective manner.

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