RESEARCH ARTICLE

In planta transformation of sorghum (*Sorghum bicolor* (L.) Moench) using *TPS1* gene for enhancing tolerance to abiotic stresses

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Abstract

An *in planta* transformation protocol for sorghum (*Sorghum bicolor* (L.) Moench) using shoot apical meristem of germinating seedlings is reported in this study. *Agrobacterium tumefaciens* strain, LBA4404 with pCAMBIA1303 vector and construct pCAMBIA1303*TPS1* were individually used for transformation. Since, the transgene is integrated into the cells of already differentiated tissues, the T_0 plants were chimeric and stable integration was observed in T_1 generation. β-Glucuronidase (GUS) expression in the seedlings and spikelets of emerging cob was the first indication of transformability in T_0 generation which was further confirmed by PCR analysis using *hpt* and *TPS1* gene-specific primers. Screening on 25 mg/L hygromycin combined with PCR analysis was used for selection of transformants in the T_1 generation. Transformation efficiencies ranged between 34–38% and 26–34% using pCAMBIA1303 vector and construct pCAMBIA1303*TPS1*, respectively. Molecular characterization of the T_2 transgenics using PCR, RT-PCR and Southern blot analyses further revealed the integration, expression and inheritance of the transgene. These results indicate the feasibility of the method to generate transgenics with pCAMBIA1303 vector and construct pCAMBIA1303*TPS1*. The abiotic stress tolerance of *TPS1* transgenics developed in the present study was evident by the ability of the transformants to tolerate 200 mM NaCl as well as higher root growth and biomass.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world. It is cultivated mostly in semi-arid tropics which are characterized by unpredictable weather, limited and erratic rainfall, high temperatures and marginal lands with problems like salinity and nutrient deficiency (Sharma and Ortiz 2000; Maqbool *et al.* 2001). Traditional breeding has been the main avenue for crop improvement for many years in sorghum, but the success achieved so far is marginal (Girijashankar and Swathisree 2009). Molecular approaches can thus be used to complement genetic engineering as they offer direct access to a vast pool of useful genes (Tari *et al.* 2013).

Sorghum has been considered as one of the plant species which is difficult to manipulate through tissue culture and transformation (Grootboom *et al.* 2010). Both, particle bombardment (Casas *et al.* 1997; Tadesse *et al.* 2003; Maheswari *et al.* 2010) and *Agrobacterium*-mediated methods for genetic transformation of sorghum (Zhao *et al.* 2000; Howe *et al.* 2006; Liu and Godwin 2012) have been reported but with low transformation efficiencies ranging from 0.18–20.7%. These methods suffer from inherent disadvantages, i.e. require sterile conditions, long period of culture, somaclonal variations etc. which are due to the *in vitro* culture of plant cells (Supartana *et al.* 2005). Therefore, *in planta* transformation methods, which require no *in vitro* culture of plant cells, have become important in recalcitrant crops like sorghum.

In planta transformation is a tissue culture independent approach to obtain fertile transformed plants. *In planta* transformation reduces time, labour, cost and most importantly, it avoids somaclonal variations (Jaganath *et al.* 2014). Several economically and agriculturally important crops have been transformed by *in planta* transformation, such as buckwheat (Kojima *et al.* 2000), mulberry (Ping *et al.* 2003) kenaf (Kojima *et al.* 2004), rice (Supartana *et al.* 2005) and wheat (Supartana *et al.* 2006) using binary vector. In all these crops, *Agrobacterium* is directed towards either the apical meristem or the meristems of axillary buds, allowing them to grow into seedlings *ex vitro*. It has been reported that the

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transformation efficiency obtained through *in planta* transformation is much higher than the conventional tissue culture based transformation (Supartana *et al.* 2006).

Synthesis of osmoprotectants, is one of the mechanisms that plants have evolved for adaptation to water deficit. Osmoprotectants include amino acids, quaternary ammonium and tertiary sulphonium compounds, polyols and sugars (Rontein *et al.* 2002). Metabolic engineering of osmoprotectants is a well-known strategy for conferring abiotic stress tolerance in crop plants. Trehalose, an osmoprotectant, a nonreducing disaccharide of glucose, plays an important physiological role as an abiotic stress protectant including bacteria, yeast, invertebrates and resurrection plants (Delorge *et al.* 2014). Trehalose, which is formed by the engineering of *TPS1* gene has been shown to stabilize dehydrated enzymes, proteins and lipid membranes efficiently as well as to protect biological structures from damage during desiccation. In *S. cerevisiae* trehalose-6-phosphate synthase (TPS) encoded by gene *TPS1* catalyzes the transfer of glucose from UDP-glucose to glucose-6-phosphate to produce trehalose-6-phosphate plus UDP. Subsequently, trehalose-6-phosphate phosphatase (TPP) encoded by the *TPS2* gene converts trehalose-6-phosphate to free trehalose (Cabib and Leloir 1958).

The introduction of exotic *TPS* encoding gene can bring about synthesis of trehalose-6-phosphate. The dephosphorylation of trehalose-6-phosphate can be catalyzed by non-specific phosphoesterase which is familiar in plant cells. Engineering trehalose accumulation into agronomically important plants is a potential approach to increase drought and salinity tolerance (Penna 2003). Transgenic plants over expressing genes coding for enzymes of the trehalose biosynthetic pathway with improved drought tolerance have been reported both in model plants like tobacco (Holmstrom *et al.* 1996; Romero *et al.* 1997; Varalaxmi *et al.* 2012) and crop plants such as potato (Yeo *et al.* 2000), rice (Garg *et al.* 2002) and tomato (Cortina and Culianez-Macia 2005).

The present study aims to develop an *in planta* transformation protocol for sorghum which is highly recalcitrant in nature. Earlier attempts to develop *in planta* transformation protocol in crop plants (Supartana *et al.* 2005, 2006) have focussed on optimization using binary vectors. In the present work, efforts have also been made to develop transgenics with *TPS1* gene for enhancing tolerance to abiotic stresses.

Materials and methods

Bacterial strain and plasmid constructs

Agrobacterium tumefaciens strain LBA4404 harbouring pCAMBIA1303 vector and pCAMBIA1303*TPS1* were used individually. pCAMBIA1303 is a binary vector having hygromycin resistance gene (*hpt*) as plant selection marker and β-glucuronidase (*gusA*) and green fluorescent protein (mg*fp5*) as reporter genes. The *TPS1* gene amplified from the strain MTCC no.174 was cloned into pTZ57R/T vector. The vector was digested with *Kpn*I, *Bam*HI and the resulting fragment was then sub-cloned into pRT100 for mobilization of promoter and terminator. The gene cassette was cloned into plant expression vector pCAMBIA1303 (Varalaxmi *et al*. 2012) and used for transformation experiments.

Transformation and generation of transgenics

One hundred μL culture of *A. tumefaciens* LBA4404 harbouring the vector pCAMBIA1303 and construct pCAMBIA1303*TPS1* individually (grown for 14 h at 28°C) was inoculated into 10 mL YEM broth containing kanamycin (50 μg/mL) and grown at 28°C until an OD_{600} of 0.8 was achieved. The cells were then centrifuged (5000 rpm/RT/10 min) and resuspended in 500 μL of fresh YEM medium and supplemented with acetosyringone (100 μM).

Sorghum seeds (*S. bicolor* (L.) Moench) cv. SPV462 were washed with *Tween-20*, rinsed 2–3 times with distilled water and then surface sterilized with 0.1% $HgCl_2$ for 10 min. Subsequently, seeds were washed 5–6 times with sterile distilled water and were aseptically germinated on wet cotton in culture bottles (50 seeds per bottle) in dark at 27°C for two days. At this stage, the meristem explants were around 2–3 mm and the root and shoot had emerged. Inoculation of the meristematic region with the resuspended *Agrobacterium* culture was carried out by using 1 mL sterile hypodermic syringes (1 mL B.D. disposable syringes, with 0.9 mm diameter needle). The inoculated seedlings were transferred to culture bottles layered with cotton at 15 seedlings per bottle and maintained at 27°C in light (85 μmol/m^2/s), under a 12 h photoperiod and were allowed to cocultivate for five days. Subsequently, the seedlings were immersed in sterile water containing 250 mg/L cefotaxime for 30 min with continuous shaking to wash off *Agrobacterium* cells, transferred to 16 cm diameter pots containing cocopit (10–15 seedlings per pot) under ambient conditions and allowed to grow for 15 days. The seedlings were then transferred into 30 × 30 cm plastic pots (three plants per pot) filled with 16 kg of sandy loamy soil (red soil : sand : farm yard manure in a ratio of 3 : 1 : 1) after acclimatization as mentioned above. The plants were selfed and grown to maturity (T_0). Appropriate untransformed controls inoculated with water were maintained following all other procedures similar to transformants. Transformation experiments were carried out with pCAMBIA1303 vector and pCAMBIA1303*TPS1*, with 150 and 300 explants, respectively, and a few of the explants were subjected to GUS histochemical assay.

Characterization of T_0 transgenics

Transient assay for GUS gene expression: Histochemical assay for transient GUS expression was performed according to Jefferson *et al.* (1987). Transient GUS expression was carried out in seedlings just before transferring it to small pots. The explants were incubated overnight at 37°C in a solution containing 50 mM sodium phosphate buffer pH 7.0, 0.1% Triton X-100, 10 mM EDTA and 1 mM 5-bromo-4-

chloro-3-indolyl β-D-glucuronide. Stable GUS expression was monitored in the spikelets of emerging cobs.

PCR analysis: Leaf sample collected at the reproductive stage was used for molecular analysis. Genomic DNA was isolated following CTAB method (Doyle and Doyle 1990). PCR analysis was carried out on putative transgenics (T_0) with pCAMBIA1303 vector using *hpt* gene-specific primers and pCAMBIA1303*TPS1* using *hpt* and *TPS1* gene-specific primers. PCR amplification was carried out using Gene AmpR PCR system 9700 (Applied Biosystems, Foster City, USA). The thermocycling conditions were initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 45 s for *hpt* and 94°C for 30 s, 55°C for 30 s and 72°C for 45 s for *TPS1* gene-specific primers and final extension at 72°C for 5 min. Genomic DNA from the untransformed control plant and plasmids pCAMBIA1303 and pCAMBIA1303*TPS1* were used as negative and positive controls, respectively.

Characterization of T_1 transgenics

Screening on hygromycin and PCR analysis: 50 and 35 seeds from each of the selected three independent T_0 transformants of pCAMBIA1303 vector (V4, V6 and V8) and pCAMBIA1303*TPS1* (L1, L2 and L3) respectively, along with untransformed control were used. The seeds were surface sterilized and germinated on half-strength MS basal medium supplemented with 25 mg/L hygromycin, a concentration which is sublethal for control sorghum seeds (Maheswari *et al.* 2010). The plants which survived hygromycin selection pressure up to nine days were transferred to pots. DNA isolated from these plants was used for PCR analysis with *hpt* and *TPS1* gene-specific primers, respectively.

Characterization of T_2 transgenics

PCR analysis: The inheritance of the transgene in the progeny of selected three T_1 transformants of pCAMBIA1303 vector and pCAMBIA1303*TPS1* was carried out using *hpt* and *TPS1* gene-specific primers, respectively.

RT-PCR analysis: Two lines from each of the three independent transgenics of pCAMBIA1303 vector (V4-3-1, V4-4-4, V6-5-3, V6-7-7, V8-5-2, V8-5-10) and pCAMBIA1303 *TPS1* (L1-1-6, L1-2-9, L2-1-3, L2-2-4, L3-1-5, L3-2-7) were selected and total RNA was isolated using Trizol method (Chomczynski 1993). First strand cDNA synthesis was carried out using Qiagen First strand cDNA synthesis kit according to the manufacturer's instructions. About 5 μg of total RNA was used for first-strand synthesis by incubating the reaction mixture at 37°C for 1 h. Second-strand synthesis was carried out using *hpt* and *TPS1* gene-specific primers.

Southern blot analysis: Southern blot analysis was carried out according to Sambrook and Russel (2001). The PCR amplified products of selected individual lines of three independent transgenics (T_2) of pCAMBIA1303 vector (V4-3-1, V6-5-3 and V8-5-2) and pCAMBIA1303*TPS1* transgenics (L1-1-6, L2-1-3 and L3-1-5) using *hpt* and *TPS1* gene-specific primers respectively were separated on a 0.8% agarose gel and were transferred onto the nylon membrane by capillary method. Hybridzation was performed at 55°C, overnight in a hybridization oven HB1000 hybridizer (UVP, Upland, USA). The 800 bp and 1431 bp *hpt* and *TPS1* gene products were used as probes. Labelling and detection was carried out using Alkaphos Direct Labelling and Detection Kit (GE healthcare cat. no. RPN3680) according to the manufacturer's instructions. GUS expression analysis in spikelets of the emerging cob of sorghum *TPS1* transgenics (T_2) was also carried out.

Seed germination on 200 mM NaCl

Tolerance of sorghum *TPS1* transgenics (T_1) to NaCl (200 mM) was tested. The seeds of *TPS1* transgenics and untransformed control were surface-sterilized and inoculated onto MS basal medium supplemented with 200 mM NaCl. Visual observations on germination as well as shoot and root growth were recorded 15 d after inoculation. The root growth of the transgenics and untransformed control were recorded at harvest.

Results

Development of transgenics

Results of the experiments conducted on *in planta* transformation, generation of transgenics and molecular confirmations for stable integration and expression are presented below. Out of the total 150 seedlings that were subjected to *Agrobacterium* inoculation using pCAMBIA1303 vector, 128 plants survived the inoculation process and were transferred to transgenic glass house. Among them, 80 plants survived, attained maturity and set seed. Out of 300 seedlings which were subjected to *Agrobacterium* inoculation using the *TPS1* gene construct, 204 plants survived the inoculation process and were transferred to the green house. Among them, 105 plants survived, attained maturity and set seed normally.

The feasibility of gene integration by *in planta* transformation protocol was ascertained based on GUS histochemical assay and PCR analysis. The blue colouration in the inoculated seedlings (figure 1, a–c) and spikelets of emerging cob (figure 1, d–f) of the pCAMBIA1303 vector and pCAMBIA1303*TPS1* transgenics upon histochemical assay suggested the introduction and expression of the *GUS* gene. No such colouration was observed in untransformed control. The lines showing stable GUS expression were checked by PCR analysis. PCR amplification of leaf genomic DNA isolated from sorghum T_0 transformants with pCAMBIA1303 vector and pCAMBIA1303*TPS1* using *hpt* gene-specific primers

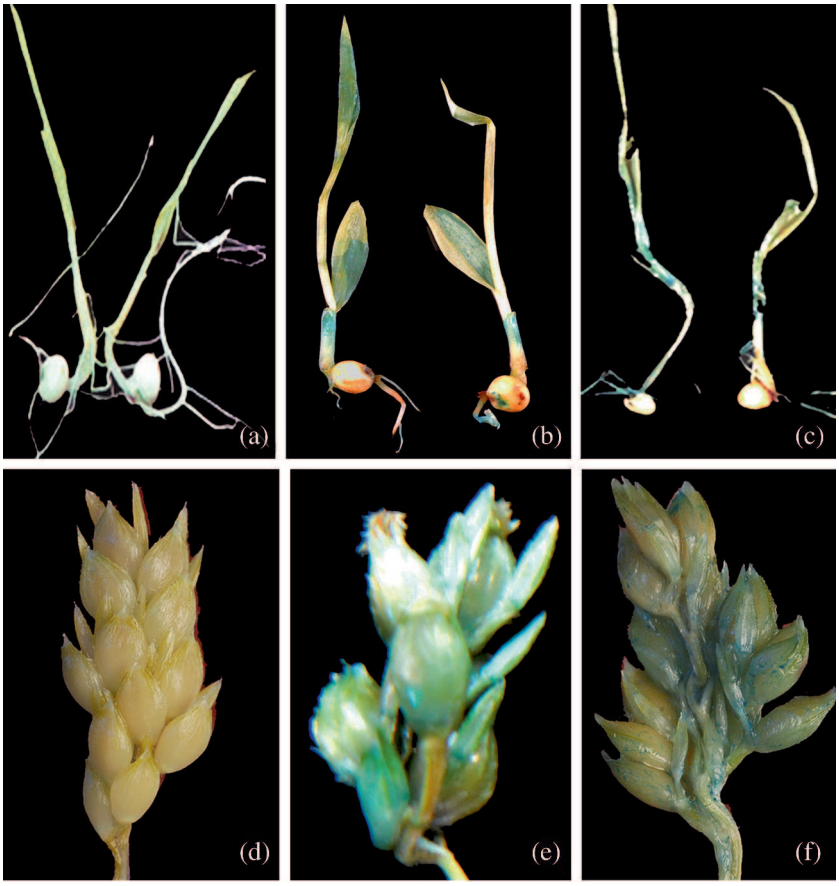

Figure 1. GUS histochemical analysis of T_0 plants. (a–c) Transient GUS activity in the infected seedlings. (d–f) Stable GUS expression in the florets. (a&d) Untransformed control, (b&e) putative transgenics with pCAMBIA1303 vector, (c&f) putative transgenics with construct pCAMBIA 1303*TPS1*.

had amplified 800 bp band corresponding to hygromycin resistance gene (figure 2, a–b). The 1431 bp band amplified using *TPS1* gene-specific primers in pCAMBIA1303*TPS1* transgenics also confirmed the presence of *TPS1* gene (figure 2c). Only those lines which were positive for above analysis were chosen for further study in the subsequent T_1 generation.

Integration and expression of the transgene in T_1 plants

Of the 50 seeds from each of the three independent primary transformants of pCAMBIA1303 vector screened on 25 mg/L hygromycin resulted in plants germinating from 22 seeds of line V4, 24 seeds of line V6 and 20 seeds of line V8. PCR analysis of the surviving plants using *hpt* gene-specific

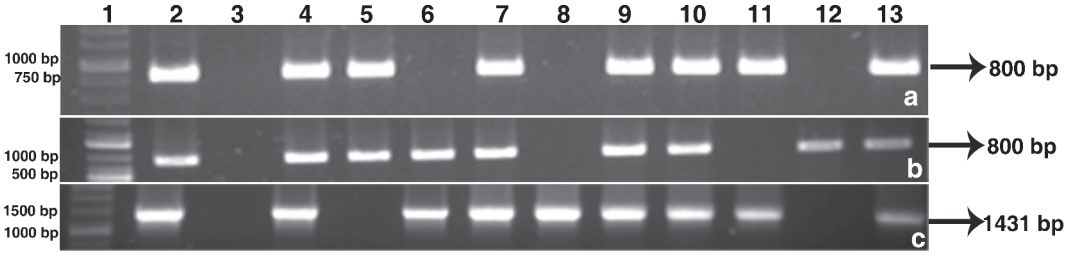

Figure 2. PCR analysis of sorghum transgenics (T_0). (a) pCAMBIA1303 vector using *hpt* gene-specific primers. (b&c) With construct pCAMBIA1303*TPS1* using *hpt* and *TPS1* gene-specific primers. Lane 1: a&c, 1 kb ladder; b, 1 kb plus ladder. Lane 2, positive control plasmid; lane 3, untransformed control; lanes 4–13, putative T_0 transgenics.

Table 1. Transformation efficiency of *S. bicolor* (L.) Monech cv. SPV462 using pCAMBIA1303 vector and pCAMBIA1303*TPS1* construct.

Total number of seeds inoculated	Number of seeds germinating on 25 mg/L hygromycin	Number of PCR positive plants	Transformation efficiency (%)*	Mean transformation efficiency (%)
pCAMBIA1303 vector				
50	23	18	36	36 ± 1.1
50	21	17	34	
50	25	19	38	
pCAMBIA1303*TPS1*				
35	15	12	34	29 ± 2.4
35	14	9	26	
35	16	10	28	

*Number of plants positive to PCR/total number of explants inoculated.

primers revealed that 18, 17 and 19 plants from each line respectively were found to be positive (figure 3a). Of the 35 seeds from each of the three independent *TPS1* transgenic plants (T_0) tested, the number of surviving seedlings on hygromycin were L1, 15; L2, 14; L3, 16. The number of lines positive to PCR analysis using *TPS1* gene-specific primers were as follows: L1, 12; L2, 9; L3, 10 (figure 3b). Only these plants were grown to maturity and seed was collected. The transformation efficiencies were calculated and are provided in table 1.

Stability of transgene in T_2 plants

PCR analysis of pCAMBIA1303 vector and pCAMBIA1303-*TPS1* transgenics (T_2) using *hpt* and *TPS1* gene-specific primers amplified 800 and 1431 bp bands in all the lines tested confirming the stable integration of the *hpt* (figure 4a) and *TPS1* genes (figure 4b). RT-PCR analysis further confirmed the integration and expression of *hpt* and *TPS1* transgenes in all the selected lines (figure 5, a&b). No such amplification was found in untransformed control. Signal generated at the expected 800 and 1431 bp positions by Southern hybridization further confirmed the transgenic nature of pCAMBIA 1303 vector and pCAMBIA1303*TPS1* transgenics, respectively (figure 5, c&d). A vector diagram showing various steps involved in *in planta* transformation protocol has been shown in figure 6.

GUS gene expression in the spikelets of the emerging cob of *TPS1* transgenics further supported the integration and expression of the transgene (figure 7). When subjected to

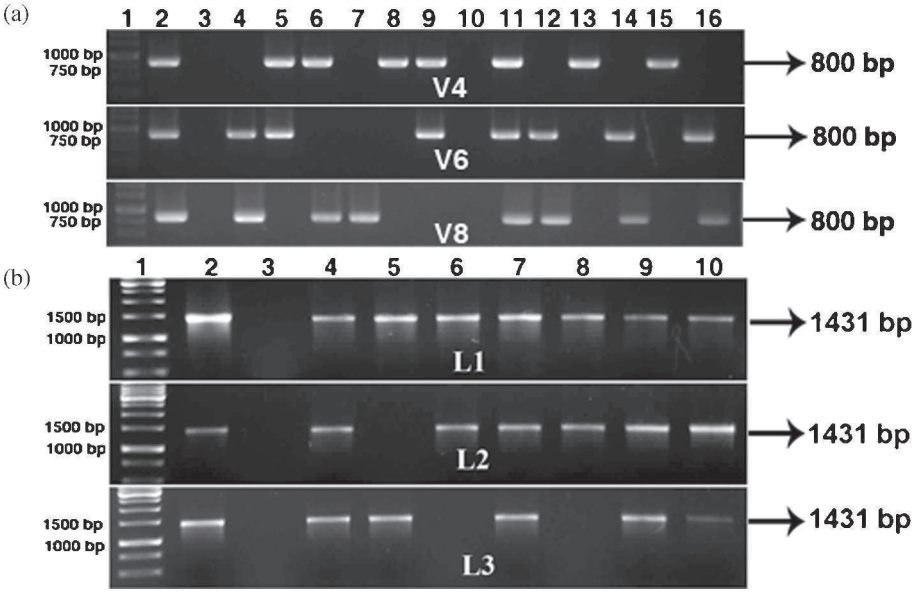

Figure 3. PCR analysis of the progeny of three independent sorghum transgenics (T_0). (a) pCAMBIA1303 vector transgenics (V4, V6 and V8) using *hpt* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lanes 4–16, T_1 transgenic lines. (b) pCAMBIA1303*TPS1* transgenics (L1, L2 and L3) using *TPS1* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lanes 4–10, T_1 transgenic lines.

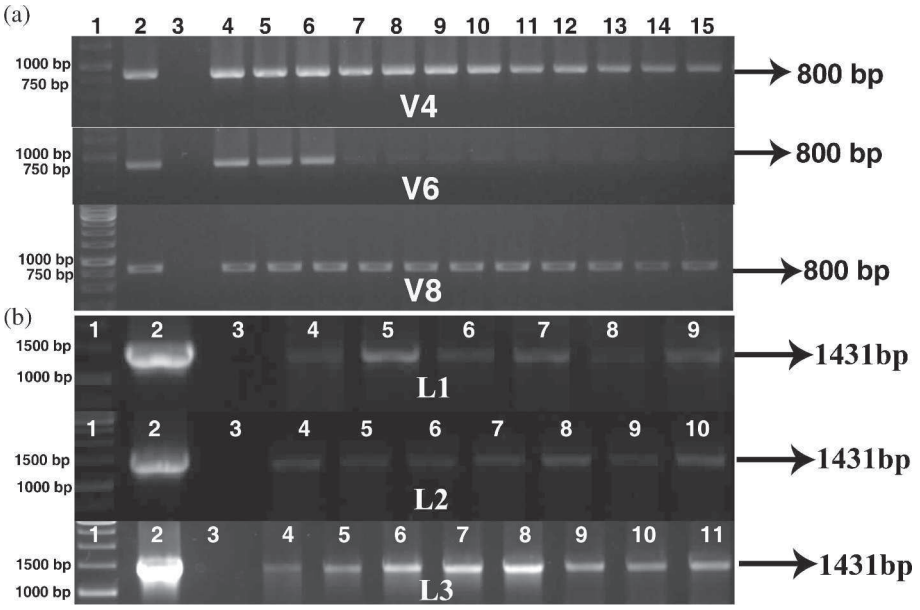

Figure 4. PCR analysis of the sorghum transgenics (T_2). (a) pCAMBIA1303 vector transgenics (V4, V6 and V8) using *hpt* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lanes 4–15, T_2 transgenic lines. (b) pCAMBIA1303*TPS1* transgenics (L1, L2 and L3) using *TPS1* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lanes 4–9, T_2 transgenic lines of L1; lanes 4–10, T_2 transgenic lines of L2; lanes 4–11, T_2 transgenic lines of L3.

200 mM NaCl treatment, seed germination as well as root and shoot growth of seedlings was higher in *TPS1* transgenics when compared to untransformed control (figure 8). At maturity, higher root growth and biomass were observed in *TPS1* transgenics when compared with untransformed control (figure 9).

Discussion

Application of transformation technology to sorghum has been impeded by its recalcitrant nature and lower transformation efficiencies (Grootboom *et al.* 2010). In the present study, a tissue culture independent *in planta* transformation

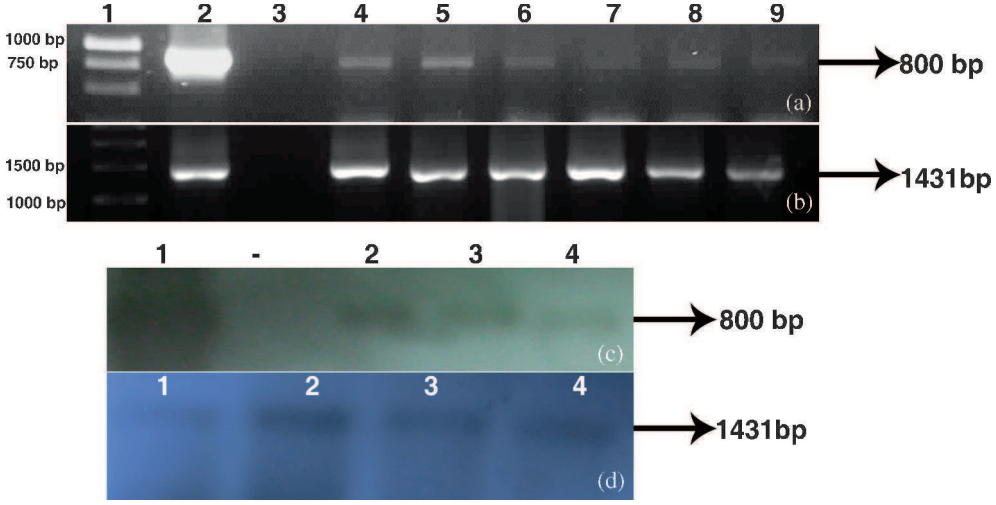

Figure 5. (a,b) RT-PCR analysis of sorghum transgenics (T_2). (a) pCAMBIA1303 vector transgenics using *hpt* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lane 4, V4-3-1; lane 5, V4-4-4; lane 6, V6-5-3; lane 7, V6-7-7; lane 8, V8-5-2; lane 9, V8-5-10. (b) pCAMBIA1303*TPS1* transgenics using *TPS1* gene-specific primers. Lane 1, 1 kb ladder; lane 2, positive control plasmid; lane 3, untransformed control; lane 4, L1-1-6; lane 5, L1-2-9; lane 6, L2-1-3; lane 7, L2-2-4; lane 8, L3-1-5; lane 9, L3-2-7. (c,d) Southern analysis of sorghum transgenics (T_2). (c) pCAMBIA1303 vector transgenics using *hpt* gene-specific primers. Lane 1, positive control plasmid; lane 2, V4-3-1; lane 3, V6-5-3; lane 4, V8-5-2. (d) pCAMBIA1303*TPS1* transgenics using *TPS1* gene-specific primers. Lane 1, positive control plasmid; lane 2, L1-1-6; lane 3, L2-1-3; lane 4, L3-1-5.

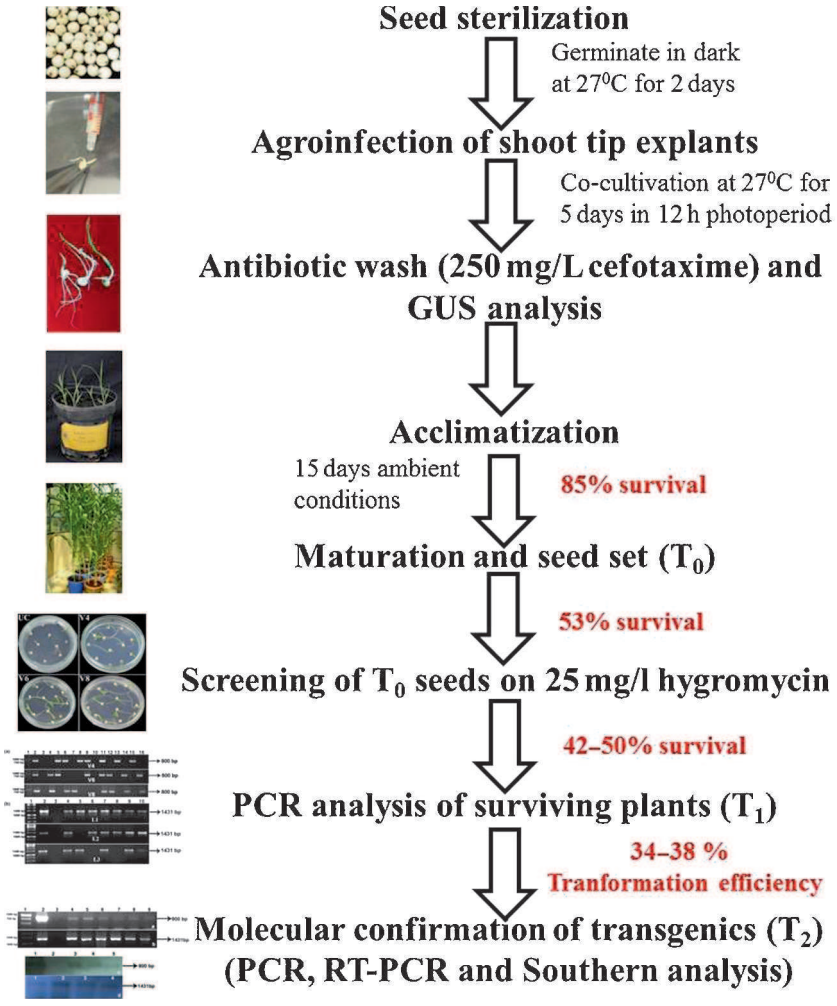

Figure 6. Vector diagram showing various steps involved in *in planta* transformation protocol for sorghum (*S. bicolor* (L.) Moench).

protocol was used to develop transformants. The most important requirement of the gene transfer approaches is high transformation frequency and efficient plant recovery which is low in case of conventional methods (Birch 1997). In the optimization of the *in planta* transformation protocol, the results indicated that the survival and recovery were higher in three-day-old explants compared to two-day-old explants. Further, a cocultivation period of five days allowed the optimal inoculation process and also helped in the establishment of the explants. No incubation of the explants in the *Agrobacterium* culture was carried out, as it is well known that with the increase in the infection time the explants turn brown and necrotic (Keshamma *et al.* 2008). Most of the explants survived the process of inoculation and a survival frequency of 85% was noted. About 80 primary transformants survived from a total of 150 seedlings. A success rate of 53% was achieved (figure 6). The developmental morphology of these plants was similar to that of seed-derived plants and they flowered normally and produced mature viable seeds.

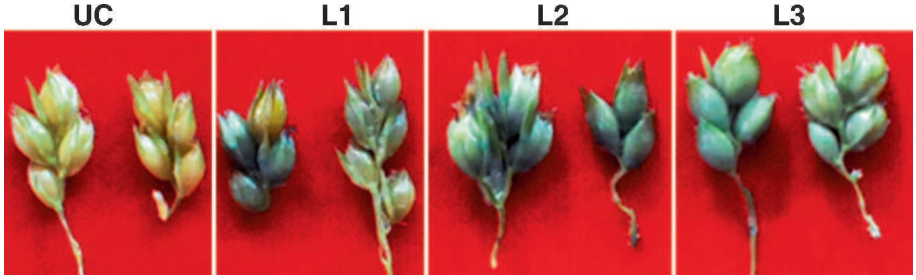

Figure 7. Stable GUS expression in the florets of three sorghum *TPS1* (T_2) transgenics (L1, L2 and L3) and untransformed control (UC).

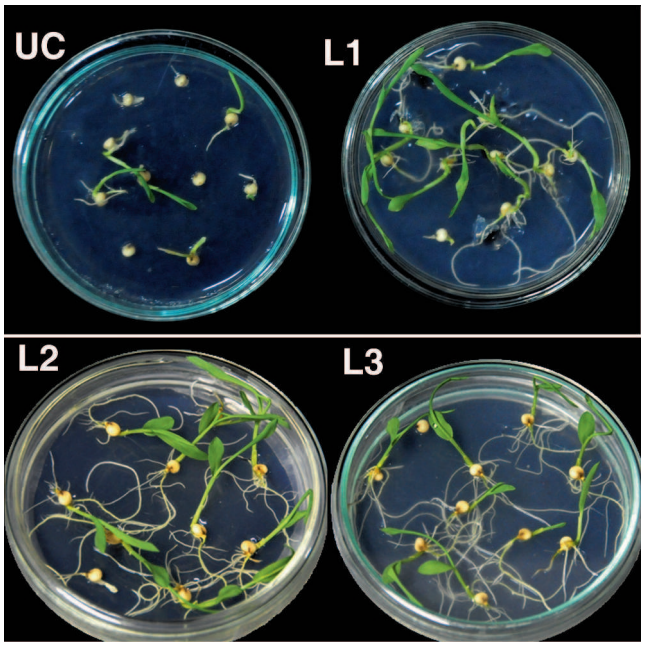

Figure 8. Seed germination of three sorghum *TPS1* (T_1) transgenics (L1, L2 and L3) and untransformed control (UC) exposed to 200 mM NaCl.

In planta transformation targets the *Agrobacterium* to the wounded apical meristem of the differentiated seed embryo. It transfers the gene into the genome of diverse cells which are already destined to develop into specific organs and also to the meristematic cells still to be differentiated, hence, the T_0 plants will be chimeric. The transgene integrated into the genome of the already differentiated cells could determine only the phenotype of the lower part of mature T_0 plants and not that of the upper part of the mature plants. In contrast, transgene integrated into the genome of yet undifferentiated cell in the meristem, which was destined to become an apical meristem of mature plants could contribute to the production of a shoot apex and hence to the phenotype of mature T_0 plants (Supartana *et al.* 2005). Thus, the transformants of the T_1 and subsequent generation would be true transformants.

The analysis of T_0 plants was carried out to assess the transformants. The blue colouration in the infected seedlings showed GUS expression indicating the transgene integration. GUS histochemical analysis of the reproductive structures can provide leads about the possibility of generating stable T_1 transformants. GUS expression in the spikelets of the emerging cobs clearly indicated that some of the T_1 seeds from these plants would produce stable transformants. PCR analysis of T_0 plants using *hpt* and *TPS1* gene-specific primers further supported the integration of the transgene.

As the number of primary transformants (T_0) produced by this method is large, a highly efficient screening protocol is needed to select putative transformants. Screening of the putative transformants on selectable marker hygromycin (25 mg/L) revealed that 42–50% of the seedlings survived were transplanted to transgenic glass house. Further, these plants were analysed by PCR analysis using *hpt* and *TPS1* gene-specific primers to eliminate the chances of escapes. About 34–38% and 26–34% of the seedlings confirmed gene integration in case of pCAMBIA1303 vector and pCAMBIA1303*TPS1*, respectively. Higher transformation efficiency in case of pCAMBIA1303 vector was observed when compared to pCAMBIA1303*TPS1*, indicating difficulty in generating the transgenics with gene construct. Similar *in planta* transformation efficiencies with binary vector based on PCR analysis has also been reported in other crops (Supartana *et al.* 2005, 2006).

In the present study, the transformation efficiencies obtained in sorghum (26–38%) are higher than that reported using conventional methods (up to 20.7%) such as particle bombardment (Casas *et al.* 1997; Tadesse *et al.* 2003; Maheswari *et al.* 2010) and *Agrobacterium* mediated approaches (Zhao *et al.* 2000; Howe *et al.* 2006; Liu and Godwin 2012). Also, most of the reports on genetic transformation in sorghum have used immature embryo explants which are season-dependent, difficult to handle and time-consuming (Gurel *et al.* 2009; Grootboom *et al.* 2010). During fertilization, *in planta Agrobacterium*-mediated transformation of flowering panicles has been reported by Elkonin *et al.* (2009) but only 1.4–2.2% survival on kanamycin has been reported.

The progeny of these plants in T_2 generation showed the amplification of the transgene in all the selected plants showing transgene inheritance. RT-PCR and Southern analyses further confirmed the expression and integration of

Figure 9. Increased root growth in the sorghum *TPS1* (T_2) transgenics (L1, L2 and L3).

the transgene. GUS expression analysis of the spikelets of the emerging cobs of *TPS1* transgenics further confirmed the inheritance of the transgene. The above analysis clearly confirmed *in planta* development of sorghum transgenics with pCAMBIA1303 vector and *TPS1* gene.

In an effort to ascertain the role of *TPS1* gene in conferring abiotic stress tolerance, seed germination assay of the sorghum *TPS1* transgenics (T_1) was carried out. Seed germination as well as root and shoot growth of seedlings were higher in transgenics of sorghum when challenged with 200 mM NaCl. At maturity, higher root growth and biomass of *TPS1* transgenics were observed when compared to untransformed control. Higher root growth of *TPS1* transgenics was earlier reported in tobacco (Romero *et al.* 1997; Varalaxmi *et al.* 2012), rice (Garg *et al.* 2002) and tomato (Cortina and Culianez-Macia 2005).

To the best of our knowledge, this is the first report of developing a tissue culture independent protocol for sorghum. In summary, here, we present the development of an efficient *in planta* transformation protocol for sorghum and also the successful generation of transgenics with *TPS1* gene with enhanced abiotic stress tolerance.

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