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Development of Plant Regeneration and Genetic Transformation System from Shoot Apices of *Sorghum bicolor* (L.) Moench

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Abstract

Development of efficient plant regeneration and genetic transformation protocols (using the Particle Inflow micro-projectile Gun and the shoot-tips as target tissue) of *Sorghum bicolor* (L.) Moench in terms of expression of the reporter gene, β-glucuronidase (*uid*A) is reported here. Two Indian cultivars of sorghum were used in the study, *viz.* M-35-1 and CSV-15. Plant regeneration was achieved from one-week-old seedling shoot-tip explants via multiple-shoot-clumps and also somatic embryos. The multiple-shoot-clumps were produced on MS medium containing BA (0.5, 1.0 or 2.0 mg/L^{-1}), with biweekly subculture. Somatic embryos were directly produced on the enlarged dome shaped expansive structures that developed from shoot-tip explants (without any callus formation) when cultured on MS medium supplemented both with BA (0.5, 1.0 or 2.0 mg/L^{-1}) and 2,4-D (0.5 mg/L^{-1}). Whereas each multiple-shoot-clump was capable of regenerating more than 80 shoots via an intensive differentiation of both axillary and adventitious shoot buds, the somatic embryos were capable of 90% germination, plant conversion and regeneration. The regenerated shoots could be efficiently rooted on MS medium containing 1.0 mg/L^{-1} IBA and successfully transplanted to the glasshouse and grown to maturity with a survival rate of 92%. The plant regeneration efficiency of both the genotypes were similar. After the micro-projectile bombardment, expression of *uid*A gene was determined by scoring blue transformed cell sectors in the bombarded tissue by an *in situ* enzyme assay. The optimal conditions comprising a helium pressure of 2200 K Pa, the target distance of 11 cm with helium inlet fully opened and the use of osmoticum have been defined to aid our future strategies of genetic engineering in sorghum with genes for tolerance to biotic and abiotic stresses.

Key words: Plant Regeneration, genetic transformation, Particle Inflow Gun, *Sorghum*

Introduction

Reports on sorghum biotechnology are very scanty (Maqbool et al. 2001). Successful development of transgenic plants requires the identification of suitable target tissue with a high plantlet regeneration frequency and efficient gene transfer protocols (Taylor and Vasil 1991). Plant regeneration in sorghum has been reported from immature embryos via shoot regeneration from callus cultures (Gamborg et al. 1977; Ma et al. 1987) and also through somatic embryos developed from embryogenic callus in sweet sorghum and grain sorghum, respectively (Mackinnon et al. 1986, 1987). Boyes and Vasil (1984) reported plant regeneration from somatic embryos through embryogenic callus from immature inflorescences in *Sorghum arundinaceum var. Sudanese* (Sudan grass). Brettel et al. (1980) reported the development of somatic embryos directly from cultured immature inflorescence explants in *S. bicolor*. Later, mature seed derived explants such as mature embryos and shoot apices of seedlings were found to be more convenient sources than immature embryos or inflorescences with comparable plant regeneration ability (Bhaskaran et al. 1988; Bhaskaran and Smith, 1988, 1989; Lusardi and Lupotto, 1990; Nahdi and de Wet, 1995). Regeneration of plantlets from culture of zygotic

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embryos or via callus cultures is a time consuming and labor intensive task. In contrast, regeneration of plantlets via multiple-shoot-tips with high efficiency rooting can easily be obtained from shoot-tip explants derived from germinated seedlings which can be obtained at any time of the year (Zhong et al. 1996 and Devi et al. 2000). Zhong et al. (1998) reported plant regeneration from shoot-tip explants in some African cultivars of *S. bicolor* via multiple shoots and also via somatic embryos.

Novel maize and pearl millet transformation systems have been developed earlier, which use particle bombardment of shoot meristems for integrative transformation and inherited expression of transgenes (Zhong et al. 1996; Devi and Sticklen, 2002). The shoot-tip contains apical initial cells and sub-epidermal cells (Medford 1992). Whereas the apical meristem cells can be multiplied for an indefinite period with or without selection and regenerated to transgenic plantlets, the sub-epidermal germinal cells can directly develop into chimeric transgenic plants from which transgenic progeny can be produced (Zhong et al. 1996). There have been a few reports on sorghum transformation with target tissue of suspension cultures (Hagio et al. 1991), protoplasts (Battraw and Hall, 1991), immature embryos (Casas et al. 1993, 1995; Kononowicz et al. 1995), calli derived from immature zygotic embryos (Zhu et al. 1998), immature inflorescences (Kononowicz et al. 1995; Casas et al. 1997) and leaf and callus using a particle inflow gun (Able et al. 1998). Devi and Sticklen (2001) reported on the standardization of parameters for efficient genetic transformation of some African cultivars using the microprojectile gun (Bio-rad).

With a long-term plan to develop transgenic sorghum (using Indian genotypes) using genes for abiotic and biotic stress tolerance, protocols for efficient *in vitro* plantlet regeneration from shoot-tip explants have been developed presently. As a first step towards the development of a genotype-independent transformation system that is based on transformation of sub-epidermal meristem cells, we delivered a marker plasmid to shoot-apical meristems of sorghum. We, therefore, describe a rapid and reliable method for particle bombardment and stable expression of the β-glucuronidase (*uidA*) gene in the shoot-tips of two Indian cultivars of sorghum with an indigenous Particle Inflow Gun.

Materials and Methods

Plant regeneration studies

Plant material, culture protocol and culture media

Dry seeds of two Indian cultivars of *Sorghum bicolor* (L.) Moench, M-35-1 and CSV-15, obtained from the National Research Centre for Sorghum, Hyderabad, India were surface-sterilized in 70% ethanol for 5 min, rinsed in sterile distilled water and washed for 30 min (with intermittent shaking) with 0.1% mercuric chloride to which a few drops of Tween-20 were added. The seeds were washed thrice with sterile distilled water and germinated in dark at 24 ± 1℃ on MS basal medium (Murashige and Skoog 1962) supplemented with 3% sucrose and 0.8% Phyta-agar (Sigma). The explants comprising shoot-tips were harvested from these aseptically germinated seedlings one week after germination. Each shoot-tip, containing an apical meristem and 2-3 leaf primordia was dissected and cultured under continuous illumination at 24 ± 1℃ on MS medium supplemented with 3% sucrose and 0.8% Phyta-agar, and supplemented with growth regulators. In preliminary *in vitro* culture experiments with different growth regulators, 2, 4-dichlorophenoxyacetic acid (2, 4-D) alone could induce compact callus, whereas BA alone could induce multiple shoots and 2, 4-D in combination with 6-benzyladenine (BA) could induce direct development of somatic embryos. Hence, to find the most efficient growth regulator combination promoting plant regeneration, the following experiments were set up with varying supplementations to the MS basal medium:

1. Control (without growth regulators)
2. 0.5 mg/L^{-1} 2,4-D
3. 0.5 mg/L^{-1} BA
4. 1.0 mg/L^{-1} BA
5. 2.0 mg/L^{-1} BA
6. 0.5 mg/L^{-1} 2,4-D + 0.5 mg/L^{-1} BA
7. 0.5 mg/L^{-1} 2,4-D + 1.0 mg/L^{-1} BA
8. 0.5 mg/L^{-1} 2,4-D + 2.0 mg/L^{-1} BA

One hundred explants were cultured on each culture medium and each experiment repeated twice (amounting to a total of 300 explants per culture medium).

Development of multiple-shoot-clumps and plantlet regeneration

All the cultures were carefully observed for signs of shoot-bud induction and the elongating leaves (from the growing shoot-tips) were regularly removed to encourage the development of the shoot-clumps. The expanding multiple-shoot-clumps were separated at every bi-weekly subculture. This was continued for 8 weeks with four passages and the data on frequency of production of multiple-shoots were recorded as the percentage of the total cultured shoot-tips producing multiple-shoot-clumps pertaining to each experiment and presented as the mean ± SE of the three replicates. Multiple-shoot-clumps developed from a single explant were maintained separately and after attaining the length of 1.5 to 2.0 cm, each individual shoot was carefully separated and

transferred to rooting medium comprising MS medium supplemented with 3% sucrose, 0.5% Phyta-agar and 1.0 mg/L^{-1} indole-3-butyric acid (IBA) and cultured at 24 ± 1℃ under continuous illumination. The rooting efficiency of the plantlets was calculated as the percentage of total rooted plantlets and presented as the mean ± SE of the three replicates. The rooted plantlets were transferred to pots with sterile soilrite and maintained in a lab at room temperature and 16 h photoperiod, covered with polythene bags for five days for acclimatization before moving them to the glass-house, where they were transplanted after 15 days to regular pots with garden soil and maintained until maturity. The percentage survival of the total transplanted plantlets was calculated after 60 days and presented as the mean ± SE of the three replicates.

Development of somatic embryos and conversion to plantlets

All the cultures were routinely inspected for the development of calli or direct somatic embryos. The somatic embryos were monitored (with subculture after every third week) and their induction response was calculated five weeks after initial inoculation as the percentage of total cultured explants producing somatic embryos and presented as the mean ± SE of the three replicates.

To study the plant conversion efficiency of the somatic embryos, uniform pieces of explants (with approximately ten globular somatic embryos) were inoculated aseptically onto the plant conversion medium (MS medium without growth regulators) and maintained at 24 ± 1℃ under 16-h photoperiod (determined after preliminary experiments). Plant conversion (germination of somatic embryos and development of the embryos into plantlets) was carefully monitored. The rate of plant conversion was calculated as the percentage of the total inoculated explants regenerating into plantlets and presented as the mean ± SE of the three replicates. To encourage better rooting and acclimatization, the regenerated plantlets were transferred to MS medium supplemented with IBA. The regenerated plantlets were then transferred to pots with sterile soilrite (1:1 mixture of soil and coconut peat) and maintained in a lab at room temperature and 16 h photoperiod, covered with polythene bags for five days for acclimatization before moving them to the glasshouse, where they were transplanted after 15 days to regular pots with garden soil and maintained until maturity. The percentage survival of all the plantlets transferred to the glasshouse was estimated after 60 days and presented as the mean ± SE of the three replicates.

Genetic transformation studies

The indigenous Particle Inflow Gun (PIG) supplied by Endeavour Enterprises, Hyderabad, India was used for genetic transformation. The PIG is based on acceleration of DNA coated tungsten particles using pressurized helium in combination with vacuum. The particles are accelerated directly by a stream of helium gas (Finer et al. 1992).

Preparation of plant material for bombardment

The shoot-tip explants from one-week old aseptic seedlings were used for particle bombardment. The shoot-tips were excised and transferred aseptically onto petriplates containing culture medium on a laminar airflow bench, 4 h prior to bombardment. 300 explants were bombarded for each experiment (100 explants in each petriplate with a total of 3 petriplates) per genotype and the experiment was repeated twice.

Plasmid

The pBluescript (6.47 kb) construct p*Act1*-F (McElroy et. al., 1990) in *Escherichia coli* contained 1.87 kb *uid*A gene, which codes for β-glucuronidase (GUS) (Jefferson 1987), driven by the rice actin-1 gene promoter (*act* 1 5', 1.44 kb) (McElroy et al. 1990) and *nos* terminator (*nos* T, 0.26 kb). The plasmid DNA was extracted with the procedure of Sambrook et al. (1989).

Preparation of plasmid DNA coated tungsten particles for bombardment

The plasmid DNA-coated tungsten particles were prepared by mixing 25 mg of tungsten particles and 250 μL of absolute ethanol and vortexed several times (at 5 min interval), spinned in a microcentrifuge (for 10 to 20 sec) before gently decanting the absolute ethanol. The tungsten particles were resuspended in sterile water, vortexed and spinned before decantation of the water. This was repeated thrice and the tungsten-water mixture was divided into 50 μL stocks. By maintaining ice-cold conditions, 10 μL of plasmid DNA was added to the 50 μL tungsten mixture along with 50 μL of 2.5M Calcium chloride and 20 μL of freshly prepared 100mM Spermidine and incubated on ice for 5 minutes. Supernatant solution was carefully removed using a Pasteur pipette and tungsten particles coated with plasmid DNA were used for bombardment (sufficient for 4 shots).

Microprojectile bombardment using Particle Inflow Gun (PIG)

The PIG (vacuum chamber) was sterilized inside by spraying with 70% ethanol. The adjustable shelf was set in the required slot. Four μL of the plasmid-DNA mixture was

spread in the center of the screen of the syringe filter-holder unit and placed on the center grid. The filter-holder was then fixed into its place. Plant material arranged in the petriplates was placed on the adjustable shelf after removing the lid. Later, this was placed on the shelf directly underneath the filter-holder (with bombardment distances of 5, 8 and 11 cm). The timer-relay bombardment switch was switched on when the vacuum pressure reached 90 Kpa. Three different helium pressures, *viz.* 1400, 1800 and 2200 Kpa were used. The procedure was repeated by removing the filter-holder each time. The effect of osmoticum on genetic transformation was also studied by culture of the target material on osmoticum (by the addition of 0.25 M sorbitol + 0.25 M mannitol to the MS medium). Following bombardment, the material was incubated at 25℃ under continuous illumination. A continuous monitoring of tissues was carried out at various stages of the experiment to check for bacterial contamination. Controls were maintained for all the parameters.

Analysis of the bombarded material

Histochemical GUS assay was carried out by a modified method of Jefferson et al. (1987). The modified histochemical assay buffer consisted of 100 mM $NaPO_4$ buffer, 100 mM Na_2EDTA, 50 mM $K_4Fe(CN)_6 \cdot 3H_2O$ and 0.1% Triton X-100 (pH 7.0). 5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (Clontech Laboratories, Palo Alto, CA, USA) was dissolved in 50% ethanol, stored at -20℃ and added to the buffer to a final concentration of 0.5 mg/mL prior to the assay.

The material bombarded with DNA coated tungsten particles (along with the control) was subjected to histochemical GUS assay at two time points. The first assay was carried out 48 h after the bombardment and the second, two weeks after the bombardment (by scoring ten petri plates from each experiment, each time). The remaining ten petri plates were maintained and regenerated to analyze the regeneration potential of the bombarded tissue. For the GUS assay, the material was immersed in the GUS substrate mixture immediately followed by vacuum treatment for 10 min, and then incubated at 37℃. The histochemical localization of GUS activity was examined under a Zeiss SV8 stereomicroscope. Chlorophyll was extracted from the material by successive incubation in 70% ethanol for 2 h and 100% ethanol overnight to facilitate better examination. The data from the experiments were evaluated as percentage of GUS positive shoot-tips (shoot-tips with at least one blue spot per explant showing transient expression of the *uid*A gene) in the total bombarded plates (for each experiment) and presented as the mean ± standard error of the replicates.

Results

Plantlet regeneration studies

Efficient *in vitro* plantlet regeneration from shoot-tip explants of sorghum was achieved in both the genotypes of sorghum through culture on MS medium supplemented with different combinations and concentrations of growth regulators (Figure 1A-E). Plant regeneration frequency via two different morphogenetic pathways, *viz.* development of multiple-shoot-clumps and development of somatic embryos has been evaluated (Table 1). The data pertaining to the frequency of production of multiple shoots, somatic embryos, rooting and percentage

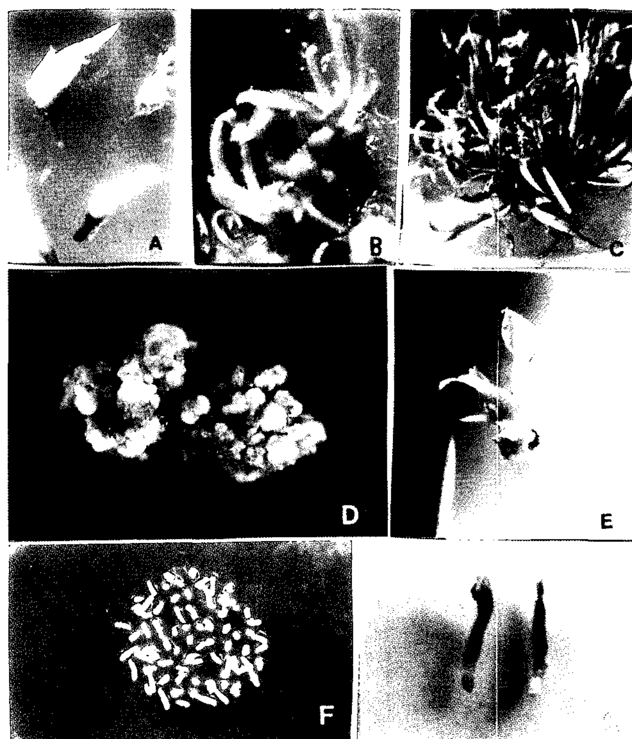

Figure 1(A-G). *In vitro* plant regeneration studies and histochemical GUS assay of the microprojectile bombarded shoot-tip explants of two sorghum genotypes.

A. to **C.** Multiple shoots produced from the shoot-tip explants of sorghum genotype: M-35-1, on MS medium supplemented with BA (2.0 mg/L^{-1}).

D. Somatic embryos developed from the shoot-tip explants of sorghum genotype CSV-15, after five weeks of culture on MS medium with BA (0.5 mg/L-1) and 2,4-D (0.5 mg/L^{-1}).

E. Healthy plantlet of sorghum genotype M-35-1, developed from a somatic embryo on plant conversion medium (MS medium without growth regulators) and sub-cultured on MS medium with IBA (1.0 mg/L^{-1}) for more profuse rooting.

F. One week-old shoot-tip explants of sorghum genotype CSV-15, ready for microprojectile bombardment.

G. Histochemical GUS assay of the bombarded explants of sorghum genotype M-35-1, two weeks after the bombardment.

survival of both the genotypes are observed to be similar. Whereas the supplementation of MS medium with only BA (0.5, 1.0 or 2.0 mg/L^{-1}) resulted in the rapid induction of profuse multiple-shoot-clumps after six weeks of incubation, the supplementation of MS medium with both 2, 4-D (0.5 mg/L^{-1}) and BA (0.5, 1.0 or 2.0 mg/L^{-1}) resulted in the direct induction of profuse somatic embryos by the fifth week. In contrast, only swelling of the explants occurred on MS basal medium (without growth regulators) and light green, compact callus developed on MS medium supplemented with 2, 4-D (0.5 mg/L^{-1}). The response of both the genotypes was similar.

Development of multiple-shoot-clumps and plantlet regeneration

Identical results of plantlet regeneration were observed in both the genotypes of sorghum.

Development of multiple-shoot-clumps, which were composed of both axillary and adventitious shoot buds, was observed on media supplemented with only BA (0.5, 1.0 and 2.0 mg/L^{-1}), where each explant initially produced 3-8 axillary buds by the third week, which in turn produced several adventitious shoot buds finally aggregating into the multiple shoot-clumps by the end of six weeks in both the genotypes of sorghum (Table 1) (Figure 1A-C.).

The multiple-shoot-clumps subsequently developed into clusters of long healthy multiple shoots (1.5 to 2.0 cm) by the fourth passage (8 weeks after initial culture of explants). The shoots were then successfully rooted on MS medium supplemented with IBA (1.0 mg/L^{-1}) and profuse roots developed by the end of two weeks. The rooted plantlets were carefully transferred to pots and moved to the glasshouse with a high frequency of survival (92%).

Development of somatic embryos and conversion to plantlets

Somatic embryos were directly produced on enlarged dome shaped expansive structures that developed from the explants after one week of culture on MS medium supplemented with both 2, 4-D (0.5 mg/L^{-1}) and BA (0.5, 1.0 or 2.0 mg/L^{-1}) in both the genotypes of sorghum (Table 1) (Figure 1. D-E). The somatic embryos were initially globular but later grew into heart shaped structures, which finally transformed to plantlets on their subculture to the plant conversion medium (MS medium without growth regulators). However, it was observed that almost all the plantlets possessed healthy shoots with relatively weaker roots. Therefore, they were transferred to MS medium supplemented with IBA (1.0 mg/L^{-1}) to encourage profuse rooting. The plantlets were then transferred to pots, moved to the

Table 1. The effect of different culture media on the relative frequency of plantlet regeneration (via multiple shoot differentiation and somatic embryo development) in two genotypes of sorghum.

Genotype	Concentration (mg/L^{-1}) of growth regulators in MS medium		[a] Frequency of production of multiple shoot clumps	[b] Frequency of production of somatic embryos	[c] Frequency of rooting on MS + IBA (1.0 mg/L^{-1})	[d] Percentage of Survival
	BA	2,4-D				
M-35-1	0.0	0.0	---	---	---	---
	0.0	0.5	(Callus)	---	---	---
	0.5	0.0	70.0 ± 1.15	---	70.0 ± 0.11	85.0 ± 1.41
	1.0	0.0	72.0 ± 1.73	---	70.6 ± 1.76	85.3 ± 0.88
	2.0	0.0	79.3 ± 0.66	---	78.3 ± 0.22	86.0 ± 1.15
	0.5	0.5	---	87.3 ± 0.66	84.6 ± 0.60	91.0 ± 0.70
	1.0	0.5	---	89.2 ± 0.55	89.3 ± 0.66	88.6 ± 0.88
	2.0	0.5	---	89.8 ± 0.62	93.0 ± 0.57	92.0 ± 0.40
CSV-15	0.0	0.0	---	---	---	---
	0.0	0.5	(Callus)	---	---	---
	0.5	0.0	71.3 ± 0.66	---	70.6 ± 0.61	84.0 ± 0.40
	1.0	0.0	75.3 ± 0.98	---	73.3 ±1.76	85.0 ± 0.41
	2.0	0.0	82.0 ± 1.15	---	81.0 ± 0.57	86.0 ± 1.41
	0.5	0.5	---	86.6 ± 1.50	86.0 ± 1.15	92.0 ± 0.81
	1.0	0.5	---	88.2 ± 0.23	89.6 ± 0.28	87.3 ± 0.88
	2.0	0.5	---	87.9 ± 0.22	92.3 ± 0.19	90.0 ± 1.52

[a] Percentage of the total cultured shoot-tip explants producing multiple-shoot-clumps, presented as mean ± S.E of the three replicates.
[b] Percentage of the total cultured shoot-tip explants producing somatic embryos, presented as mean ± S.E. of the three replicates.
[c] Percentage rooting and acclimatization of the total cultured plantlets, presented as mean ± S.E. of the three replicates.
[d] Percentage survival of the total transplanted plantlets after sixty days of growth in the glasshouse, presented as mean ± S.E. of the three replicates.

glass house and grown to maturity with 90% survival.

Genetic transformation studies

The shoot apical meristems were bombarded with tungsten particles coated with p*Act1*-F plasmid DNA containing 1.87 kb *uid*A gene driven by rice actin1 promoter (*Act1*) and *nos* terminator. Amongst the various combinations of helium pressures and target distances with and without osmoticum, the parameters comprising the helium pressure 2200 k Pa, the target distance of 11 cm with helium inlet fully opened and the use of osmoticum were found to be most suitable for producing maximum number of shoot-tips with GUS positive blue spots in the epidermal tissue of the meristem after histochemical GUS assay (Figure 1F-G and Tables 2 and 3). The GUS assay was carried out after two time points (48 hours and 2 weeks) and the number of blue spots in the bombarded explants decreased with the increase in culture duration after bombardment.

Discussion

Plantlet regeneration studies:

In vitro regeneration of plantlets from shoot apical meristems via multiple shoots and somatic embryos has been achieved in two Indian cultivars of sorghum. Genotype independent regeneration protocols have been standardized by simply supplementing the MS medium with BA and also with both BA and 2, 4-D. The results of the current investigation are consistent with those of Zhong et al. (1998). In the present study, multiple shoots were produced in abundance on culture medium supplemented with BA. The latter is in agreement with the report of Bhaskaran and Smith (1989), who stated that multiple shoots could be induced with high concentration of cytokinin (BA). The present observation also agrees well with several earlier reports on cereals (Zhong et al. 1992, 1998; Zhang et al. 1996; Devi et al. 2000, 2003). Zhong et al. (1998) explained the morphogenetic

Table 2. Optimization of particle gun delivery system. Effects of pressure of helium gas and bombardment distance on transient GUS gene expression[a] in shoot-tip explants of two genotypes of sorghum.

Pressure of helium gas (Kpa)	Bombardment distance (cm)	GUS expression after 48 h		GUS expression after two weeks	
		M-35-1	CSV-15	M-35-1	CSV-15
1400	5	9.4 ± 0.2	8.2 ± 0.2	1.5 ± 0.6	1.0 ± 0.2
	8	15.0 ± 1.5	14.3 ± 0.2	4.5 ± 0.2	2.1 ± 0.2
	11	13.2 ± 0.4	12.0 ± 1.1	1.1 ± 0.2	1.1 ±0.5
1800	5	11.6 ± 1.1	9.3 ± 0.3	2.2 ± 0.1	1.7 ± 0.2
	8	29.0 ± 0.5	29.3 ± 0.8	2.1 ± 0.2	2.2 ± 0.1
	11	31.6 ± 0.3	34.2 ± 0.4	3.0 ± 0.4	3.5 ± 0.4
2200	5	12.4 ± 0.4	11.8 ± 0.7	1.2 ±0.2	1.8 ± 0.4
	8	30.3 ± 0.8	30.0 ± 1.1	5.0 ± 0.1	4.7 ± 0.5
	11	36.6 ± 0.8	39.0 ± 0.5	6.2 ± 0.1	5.8 ± 0.6

[a] Data represents the mean ± S.E values of the percentage of GUS positive shoot-tips (with at least one blue spot within the meristem) in the total of 9 bombarded petriplates containing a total of 900 explants for each experiment (100 explants / petriplate) per genotype pertaining to the three replicates.

Table 3. Evaluation of use of culture medium with osmoticum on transient GUS gene expressiona at optimum bombardment conditions[b] in shoot-tip explants of two genotypes of sorghum.

Genotype	Osmoticum	GUS expression	
		48 h	2 weeks
M-35-1	Presence	38.6 ± 0.8	6.0 ± 0.1
	Absence	3.9 ± 0.3	0.0 ± 0.0
CSV-15	Presence	39.0 ± 0.5	5.8 ± 0.4
	Absence	4.1 ± 0.1	0.0 ± 0.0

[a] Data represents the mean ± S.E values of the percentage of GUS positive shoot-tips (with at least one blue spot within the meristem) in the total of 9 bombarded petriplates containing a total of 900 explants for each experiment (100 explants / petriplate) per genotype pertaining to the three replicates.
[b] Helium pressure: 2200 Kpa; Bombardment distance: 11 cm.

plasticity of the sorghum shoot apices that could be induced to develop axillary shoots, adventitious shoots or somatic embryos by simply manipulating the concentration of BA and 2, 4-D in the culture medium. Our results proved that addition of cytokinin (BA) induced highly efficient multiple shoot development, and further addition of low level of auxin (2, 4-D) and cytokinin (BA) induced direct somatic embryogenesis in contrast to the report of Zhong et al. (1998), where the inclusion of both BA and 2, 4-D in the culture medium triggered a higher frequency of adventitious shoot formation and also embryogenesis. However, the change of morphogenetic pathways in response to the supplemented growth regulators observed presently supports the view of Zhang et al. (1996). The results show that the present plant regeneration system is genotype independent with a high survival percentage.

Whereas the current results report the direct development of somatic embryos without an intervening callus phase, followed by plant conversion, several earlier reports have dealt with the regeneration of plantlets through the somatic embryos produced through embryogenic callus (Bhaskaran and Smith, 1989; Lusardi and Lupotto, 1990). However, as far as the production of somatic embryos is concerned, our results indicate that they could be produced (directly) on a culture medium supplemented with auxin (2, 4-D) and cytokinin (BA), and is in agreement with Bhaskaran and Smith (1989).

Very slight genotypic differences were observed in case of frequency of production of multiple shoots as well as for the production of somatic embryos, suggesting that the regeneration system is genotype independent.

Genetic transformation studies

The Particle Inflow Gun was used in the present study to bombard the target meristems with the *uid*A construct. The bombardment parameters (PIG) were optimized using expression of *uid*A gene as reported in maize embryogenic suspension cultures (Finer et al. 1992) and sorghum leaf and callus cultures (Able et al. 1998). The present results agree with those of Finer et al. (1992), Rathus et al. (1996) and Able et al. (1998) with respect to the optimum parameters. Further, the results were found to be genotype independent. The number of blue spots in the bombarded explants decreased with the increase in culture duration after bombardment, similar to the reports of Devi and Sticklen, (2001, 2002). This indicated that most blue spots are due to transient expression of the reporter gene. Transient assays could be used to optimize some conditions for stable transformation of sorghum. The efficiency of foreign gene delivery by particle bombardment depends on several factors like physiological state and morphological character of cells, DNA carrying ability of the particles, effective penetration of the particles into the cells without excessive damage, and the degree of expression of the foreign genes in the bombarded cells (Wang et al. 1998). Slight genotypic difference observed, in case of the transient expression, may be due to the inherent characteristics of the cells, which may make them unresponsive for transient expression. The next step of this study will involve the establishment of a stable transformation system of sorghum by transfer of genes for biotic and abiotic stress tolerance. Presence of osmoticum was more beneficial in case of both genotypes and is similar to the report of Casas et al. (1993).

The rice *Act1* promoter that is known to cause a high level of *uid*A expression in transformed rice and maize cells (McElroy et al. 1990; Zhang et al. 1991) efficiently controlled the transient expression of *uid*A gene in cultured shoot-tip explants of sorghum in the present study. The high levels of transient expression seem to be caused by the constitutive expression of the rice *Act1* (McElroy et al. 1990; Zhang et al. 1991). These findings suggest that the rice *Act1* promoter is an efficient and useful in transformation of monocotyledonous crops (Takumi et al. 1997). An efficient genetic transformation system has been standardized in sorghum presently with the highly regenerable *in vitro* developed multiple shoots and somatic embryo cultures differentiated from shoot-tip explants of Indian sorghum genotypes by using an indigenous PIG.

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