INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–1092/2013/15–6–1157–1162
http://www.fspublishers.org

Full Length Article

Genotypes, Explants and Growth Hormones Influence the Morphogenesis in Pakistani Sorghum (*Sorghum bicolour*): Preliminary Field Evaluation of Sorghum Somaclones

Muhammad Zarif[1], Bushra Sadia[1*], Riaz Ahmad Kainth[2] and Iqrar Ahmad Khan[1]
[1]*Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Fodder Research Substation, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan*
*For correspondence: bushrauaf@gmail.com

Abstract

Present study reports the establishment of a robust *in vitro* regeneration protocol for Pakistani sorghum lines, Pak China-1 and Sandal Bar. Mature seeds and immature inflorescences were assessed for morphogenesis. MS medium with 2,4-D and kinetin induced callus from both the explants. Whereas, MS fortified with NAA (0.5 mg/L) + kinetin (1 mg/ L) + Casein hydrolysate (400 mg/ L) proved the best regeneration medium for immature inflorescence-derived calli. *In vitro* regeneration from seed-derived calli was the maximum on LS medium supplemented with IAA (2 mg/ L) + BA (2 mg/ L). The axenic regeneration and establishment of sorghum somaclones in the glasshouse completed within 20-22 weeks. Two hundred somaclones have been shifted to the field; their preliminary evaluation indicated variations for some economically important traits including no. of tillers, seed yield, leaf area and stem thickness. Further evaluations are underway. © 2013 Friends Science Publishers

Keywords: *Sorghum bicolor*; Tissue culture; 2,4-D; Immature inflorescence; Somaclones

Introduction

Sorghum belongs to genus of grasses raised for grain and fodder. It is a highly valuable diverse crop, cultivated in tropical, subtropical, temperate and semi-arid regions of the world. Being the world's fifth most important cereal crop, it serves as the dietary staple for more than 500 million people worldwide (Liu and Godwin, 2012). Global area under sorghum crop is over 44.91 m ha producing 65.53 m tons of grain with an average of 1.4 tons/ha (FAO, 2010). About ninety percent of the planted area is in developing countries. Sorghum is a hardy crop which can withstand extreme drought and shows a generous crop stand on marginal lands with poor nutrition. Once used for table syrup and livestock feed, sorghum is now ready to play a key role in renewable energy worldwide (Belton and Taylor, 2004). Depending on the characteristics, sorghum can be grouped as grain, forage, high-tonnage and sweet sorghum types.

Sorghum is the fourth important cereal crop of Pakistan after wheat, rice and maize, and the second leading fodder after Egyptian clover or berseem (*T. alexandrinum* L.). The production and yield of Pakistani fodder sorghum is quite low compared to other sorghum producing countries of the world (Durrishahwar *et al*., 2012). In this context, traditional breeding needs to be supplemented with target-oriented biotechniques to boost sorghum production. Being one of the most important annual grasses; its improvement for yield and quality traits can greatly reduce the risk of inadequate forage production in summer.

Until recently, the exploitation of plant biotechnology in sorghum received less attention as against other cereals, owing to the recalcitrance of this crop to *in vitro* culture protocols (Prasad, 2012). However, the crop gained renewed global interest as a leading biofuel crop.

To date, various techniques of biotechnology including tissue culture (Sadia *et al*., 2010), genetic transformation (Liu and Godwin, 2012), molecular markers (Iqbal *et al*., 2010), genomics (Paterson *et al*., 2009) and proteomics (Ngara *et al*., 2012) have been successfully exploited in sorghum. The efforts are underway to generate world's first bio fortified golden sorghum (http://biosorghum.org/). Despite these achievements, the reports on exploitation of biotechniques to Pakistani sorghum are very scarce, most of these are limited to genetic fingerprinting (Akram *et al*., 2011).

Present study is an attempt to establish a highly efficient and reproducible protocol for *in vitro* propagation of Pakistani sorghum. The established protocol will provide a baseline for the exploitation of genetic manipulation techniques in an effort to develop high yielding sorghums.

To cite this paper: Zarif, M., B. Sadia, R.A. Kainth and I.A. Khan, 2013. Genotypes, explants and growth hormones influence the morphogenesis in Pakistani sorghum (*Sorghum bicolour*): preliminary field evaluation of sorghum somaclones. *Int. J. Agric. Biol*., 15: 1157–1162

Materials and Methods

The plant material comprised of two improved sorghum lines viz., Sandal Bar and Pak China-1 (PC-1), developed at Fodder Research Sub Station, Ayub Agricultural Research Institute (AARI), Faisalabad. Immature inflorescences and mature seeds of sorghum were used as explants.

Explant Preparation and Sterilization

Immature inflorescences were collected from 60 to 70 day old field grown sorghum plants. The outer leaves were removed and the shoots were surface sterilized with 70% ethanol for 30 sec in the air flow cabinet. The 3 mm segments of the sterilized immature inflorescences were used throughout. Dried mature seeds were washed in running tap water with a few drops of detergent for 30 min. Seeds were immersed in 75% ethanol for 3 min and then in 10% sodium hypochlorite for 15-20 min in airflow with continuous shaking followed by four-five rinses with autoclaved reverse-osmosis water.

Culture Media Used

Callus induction and proliferation: MS medium (Murashige and Skoog, 1962) supplemented with varying levels of 2,4-D (1,2,4, and 6 mg/L) and 0.2 mg/L of kinetin was used.
Plant regeneration: MS and LS (Linsmaier and Skoog, 1965) media supplemented with different levels of IAA, NAA, BAP, kinetin, casein hydrolysate and TDZ were used (Table 1).
Rooting of *in vitro*-regenerants: MS, ½ strength MS alone and in combination with NAA and IBA were used in different concentrations. All culture media contained 30 g/L sucrose and 8 g/L agar. The pH of media was adjusted to 5.8 before autoclaving at 121°C for 15 min.

Callus Induction and Proliferation

Sterilized mature seeds and immature inflorescence segments were cultured on callus induction media. Aliquots (20 mL) of each medium were dispensed into 9 cm diametere Petri dishes. Five to seven seeds and 8-10 inflorescence segments were cultured per dish per medium. Each medium was replicated ten-folds and dishes were sealed with parafilm. The cultures were incubated at 22 ± 2ºC under a 16 h photoperiod (19.5 µmol m^{-2} s^{-1}, Cool White fluorescent tubes). Calli were transferred to respective fresh media 28 d post-initiation and maintained under the same growth conditions. Different callogenic parameters were recorded including days to callus initiation, percent callus induction and callus morphology.

Callus-to-plant Regeneration, Rooting and Hardening

The proliferated embryogenic calli 8-12 weeks after culture were transferred to MS- and LS-based different regeneration media. Each treatment was repeated 10 times. The cultures were incubated under same conditions as mentioned for callogenesis. Data on various regeneration traits were recorded. Callus-derived sorghum regenerants (2-3 cm long) were transferred individually to rooting media. Sorghum *in vitro* plants with fully developed roots were taken out of culture media, washed thoroughly in running tap water and shifted to disposable glasses having well-rotted peat and muck soil. The plants were covered with polyethylene bags and the vents were opened gradually in 8-10 days to avoid evapotranspirational losses of water. Later, these were shifted to bigger pots in the glasshouse, for root proliferation.

Parameters Recorded for Callogenesis and Plant Regeneration

The data were collected for the following parameters:
Days to callus initiation: The days required to initiate callus by different explants cultured on callus induction media.
Percent callus induction: Recorded after 6 weeks of incubation as number of explants inducing callus divided by total number of explants cultured on callus induction media.
Regeneration frequency: Data for regeneration frequency were recorded as number of callus segments producing shoots per total number of calli shifted to shoot induction media.
Number of shoots per explant: Total number of shoots produced by each callus segment on shoot induction media.
Rooting frequency: Recorded as number of individual shoots producing roots divided by total number of shoots transferred to root induction media.

Transplantation of Sorghum Somaclones in the Field

The *in vitro*-derived regenerants of sorghum were taken to the field as R0 generation for further evaluation of morphological traits and their inheritance pattern. R0 somaclones were selfed to produce R1 generation. Data for various morphological traits of somaclones and their respective parent lines were recorded.

Statistical Analysis

The *in vitro* experiments were carried out following Completely Randomized Design (CRD) in a factorial arrangement with three replications per treatment per explant. The means and standard deviations were computed for each treatment. The data collected were analyzed statistically through ANOVA using LSD test at 5% level of significance for comparing the differences among treatments.

Table 1: Plant regeneration media for seeds and immature inflorescence-derived calli of sorghum

Regeneration media	Media Composition	
	For seed culture	Immature inflorescence culture
RM_1	MS_0	MS_0
RM_2	LS+2 mg/l IAA+2 mg/l BA	MS+0.5 mg/L NAA+1 mg/L kinetin +400 mg/L casein hydrolysate
RM_3	LS+1 mg/L IAA+2 mg/L BA	LS+1 mg/L IAA+2 mg/L BA
RM_4	MS+0.5 mg/L IAA +2 mg/L BA+ 0.5 mg/l kinetin	MS+1 mg/L NAA+1 mg/L kinetin + 400 mg/L casein hydrolysate
RM_5	MS+1 mg/L IAA+3 mg/L BA	MS+3 mg/L BAP+1mg/l TDZ
RM_6	—	MS+2 mg/L BA+ 0.5mg/l kinetin

RM= Regeneration media

Results

Callus Induction Studies

Effect of sorghum explants and genotypes on callogenesis: Callus initiation recorded in both the explants of PC-1 and Sandal Bar when cultured on MS + 2,4-D supplemented media. The callogenesis started early in PC-1 for both seeds and immature inflorescence explants than Sandal Bar (Table 3). Friable calli of yellow to greenish shades were induced in both the lines. In case of seed explants, PC-1 produced more callus than Sandal Bar. Similarly, with immature inflorescence explants, PC-1 gave higher callus values than Sandal Bar. Overall, PC-1 proved efficient callus producer sorghum line with higher callus induction frequency as against 45.5% for Sandal Bar. Higher callus induction response was observed in immature inflorescences as compared to seeds.

The callus induction time from seed and immature inflorescence explants of both sorghum lines varied with the 2,4-D concentration. The lowest and the highest levels of 2,4-D (1 and 6 mg L^{-1}) delayed callus induction. Overall, moderate auxin level (4 mg L^{-1}) supported early callus induction and higher callus induction percentage in both explants of the two sorghum lines (Table 3 and 4).

Morphological Characterization of Seed and Immature Inflorescence-derived Calli of Sandal Bar and PC-1

Wide variations were observed in the degree of callus formation, callus color and texture on different callus induction media (Table 5). Immature inflorescences produced green and yellow calli. Callus texture changed from watery to compact to friable with increasing 2,4-D concentration. Callus quantity was higher at 4 mg/L of auxin. Seed explants however, produced creamy yellow colored friable calli on most of 2,4-D levels. Callus quantity was low at 1 and 6 mg/L and the highest at 4 mg/L of 2,4-D.

Callus-to-Plant Regeneration in Sorghum

Response of sorghum lines to regeneration: Shoot regeneration began 2-3 weeks after culture on all the regeneration media. While comparing both the lines, PC-1 showed higher regeneration response than Sandal Bar (Table 6). In case of seed-derived calli, PC-1 gave higher average regeneration frequency as against Sandal Bar (Fig. 1). The immature inflorescence-derived calli started organogenesis in Sandal Bar and PC-1 on all the regeneration medium combinations, with higher number of shoots in PC-1 (Fig. 1).

Shoots from both sorghum lines started rooting one week after transfer to all the rooting media. PC-1 shoots showed high rooting frequency (62.25) than Sandal Bar (57.5%). Rooting medium RIM_2 produced the maximum roots (81.00%), followed by RIM_4 and RIM_3 (69.50 and 59% root induction frequency, respectively). While, MS_0 was the least effective rooting medium (Table 7). The study indicated that initial callus induction medium played a decisive role in sorghum plant regeneration. The level of

Table 2: Rooting media for seeds and immature inflorescence callus-derived shoots of sorghum lines

Rooting media	Media Composition
RIM_1	MS_0
RIM_2	½MS
RIM_3	½MS +NAA (1 mg/L)
RIM_4	½MS +NAA (1 mg/L) +I BA (2 mg/L)

Table 3: Response of Sandal Bar and PC-1 for callus induction time using seeds and immature inflorescence explants

Media Treatments	Callus induction time	
	Seeds	Immature inflorescences
MS_0	0.00	0.00
MS+2,4-D 1mg/l	18.00a	13.40a
MS+2,4-D 2 mg/l	13.00a	15.50a
MS+2,4-D 4 mg/l	10.50b	11.50b
MS+2,4-D 6 mg/l	14.00b	18.00b
Mean	11.1	11.68

Values are means of 50 observations per treatment per explants. All media treatments were supplemented with 0.2 mg/L of kinetin

Table 4: Effect of 2,4-D on percent callus induction in sorghum

2,4-D level	Percent callus induction			
	Seeds		Immature inflorescence	
	Sandal Bar	PC-1	Sandal bar	PC-1
0 mg/L	0.00	0.00	0.00	0.00
1 mg/L	9.90e	27.50f	48.00h	59.70f
2 mg/L	44.90d	73.90b	64.80e	85.70b
4 mg/L	65.00c	79.90a	79.70c	90.60a
6 mg/L	29.90f	48.00d	54.80g	71.90d
Mean	29.94b	45.86a	49.46	61.58b

Values are means of 50 observations per explant per treatment

Table 5: Morphological characterization of immature inflorescence- and seed-derived calli of sorghum lines Sandal Bar and PC-1 as influenced by different levels of 2,4-D

Treatments (MS+2,4-D mg/L)	Callus Morphology (Immature inflorescences)						Callus Morphology (Seeds)					
	Sandal Bar			PC-1			Sandal Bar			PC-1		
	Quantity	Color	Texture	Quantity	Color	Texture	Quantity	Color	Texture	Quantity	Color	Texture
0	–	–	–	–	–	–	–	–	–	–	–	–
1	++	Greenish	Watery	++	Light Green	Watery	+	Yellowish	Watery	+	Light yellow	Watery
2	+++	Light Green	Friable	+++	Yellowish	Compact	++	Light yellow	Friable	+++	Yellowish	Friable
4	++++	Light Green	Compact	++++	Light green	Compact	++++	Creamy	Friable	++++	Creamy	Compact
6	++	Light Yellow	Friable	++	Yellow	Friable	+	Yellow	Friable	++	Yellow	Friable

Table 6: Regeneration from inflorescence- and seed-calli of sorghum lines Sandal Bar and PC-1 as influenced by different media

Regeneration media	Regeneration frequency			
	Sandal Bar		PC-1	
	Immature inflorescence	Seeds	Immature inflorescence	Seeds
RM_1	0.00g	0.00g	0.00g	0.00g
RM_2	73.00b	25.00c	85.00a	35.00a
RM_3	58.00c	18.00d	70.00b	28.20b
RM_4	37.14e	9.00e	53.49d	19.00d
RM_5	28.00f	2.00f	40.17e	9.00e
RM_6	12.00g	-	25.00f	-
Mean	34.69b	10.80 b	45.61a	18.24a

Values are means of 50 observations / explant /treatment/ line

Table 7: Rooting frequency of sorghum lines Sandal Bar and PC-1 assessed on different rooting media

Rooting media	Rooting frequency		Treatment Mean
	Sandal Bar	PC-1	
MS_0	25.00 ± 0.20h	35.00 ± 0.04g	30.00b
RIM_2	56.00 ± 0.60f	62.00± 0.25e	59.00b
RIM_3	78.00 ± 1.05b	84.00 ± 0.76a	81.00a
RIM_4	71.00 ± 0.90c	68.00 ± 0.80d	69.51a
Mean	57.50 ± 0.69b	62.25 ± 0.46a	

Values are means of 50 observations per explant per treatment

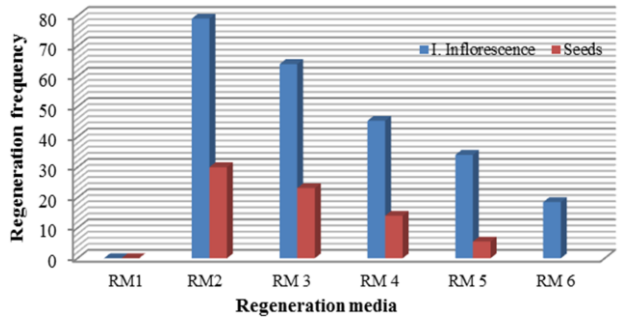

Fig. 1: Plant regeneration response of sorghum explants on different regeneration media

2,4-D during callus induction showed a strong carry over effect on later stage of plant regeneration from these calli. Sorghum calli from both the explants induced on MS + 2 mg/L of 2, 4-D regenerated more plants, followed by MS medium fortified with 4 and 1 mg/L of 2,4-D, respectively. The calli produced on MS medium having the highest concentration of 2,4-D showed the poor regeneration response (Fig. 2).

Sorghum shoots with fully developed roots were acclimatized in pots and then shifted to field. PC-1 produced higher number of plants per explant on an average as against Sandal Bar (Table 6).

Transplantation of Sorghum Regenerants in the Field

The *in vitro*-derived potted sorghum plants were planted in Experimental area of Fodder Research Sub Station, Ayub Agricultural Research Institute, Faisalabad. The row-row distance was 30 cm and each row was 3 m long. Standard crop production technology as needed for sorghum crop was used in the experiment. One hundred and twenty somaclones were field transplanted. The R0 generation showed satisfactory growth during reproductive phase. After harvesting panicles, the field was irrigated and fertilized. Most of the harvested sorghum stems sprouted and produced multi tillers. The plants are under morphological assessment in the field. The comparison of first generation of *in vitro*-regenerated plants of PC-1 and Sandal Bar for different morphological traits is presented in Table 8. Field performance of sorghum somaclones was similar to their respective parents for most of the morphological traits. Whereas, variations were recorded for few economically important traits including no. of tillers, seed yield, leaf area and stem thickness. Somaclones of both PC-1 and Sandal Bar produced multi tillers. This is a good note since multi tillering is an economically beneficial trait. Data also indicated more leaf area and reduced stem thickness in somaclones compared to their parents. Likewise, the somaclones proved better seed yielders than the respective parents. Further evaluation of somaclones is in progress.

Discussion

The efficient *in vitro* culture system is of prime importance in the exploitation of sorghum genetic manipulation techniques. In this regard, several factors play a key role from explant selection to the transplantation of regenerants in the field. Sorghum tissue culture is highly genotype-dependent, so the selection of tissue culture-responsive genotypes helps establish a robust

Table 8: Preliminary field evaluation of sorghum somaclones and their respective parents for different morphological traits

Traits	PC-1		Sandal Bar	
	Parent plants	Somaclones	Parent plants	Somaclones
Plant height (cm)	180 ± 0.91	200 ± 2.39	195 ± 3.20	220 ± 3.65
No. of leaves/ plant	20 ± 0.67	19 ± 0.61	20 ± 0.58	20 ± 2.51
Leaf colour	Dark green	Dark green	Dark green	Dark green
No. of tillers/ plant	0.00± 0.00	5.1 ± 0.5	0.00± 0.00	5.00 ± 0.40
Stem	Juicy	Juicy/ sweet	Juicy	Juicy/ sweet
Panicle shape	Compact	Compact	Compact	Compact
Maturity	Stay green/late	Stay green/late	Stay green/late	Stay green/late
Panicle size (cm)	16.1 ± 0.64	21.1 ± 2.30	14.2 ± 3.80	19.2 ± 5.40
Leaf area (cm^2)	285 ± 4.17	315 ± 6.17	290 ± 5.54	320 ± 11.33
Stem thickness (cm)	0.1 ± 0.016	0.083 ± 0.012	0.1 ± 0.014	0.097 ± 0.01
Seed yield (Kg/acre)	1140 ± 13.81	1400 ± 15.29	1182 ± 10.55	1440 ± 12.77

tissue culture protocol in short time (Liu and Godwin, 2011). In the present case however, there is no report of *in vitro* culture studies of Pakistani sorghum genotypes. So, two promising lines PC-1 and Sandal Bar were used. Selection of appropriate explants affects the *in vitro* protocol optimization. We used immature inflorescences the most widely used sorghum explants (Mani and Pola, 2003; Gupta *et al.*, 2006; Jogeswar *et al.*, 2006; Anbumalarmathi and Nadarajan, 2007) along with mature seeds, also exploited in earlier investigations (Zhao *et al.*, 2008; Arulselvi and Krishnaveni, 2009). The composition of callus induction medium greatly influenced the morphology and texture of sorghum callus. Callus induction is favoured by low levels of 2,4-D in majority of cereals, this fact has also been reported for sorghum by Manjula *et al*. (2000) and Pola *et al*. (2011). We also observed the efficient callogenesis at 4 mg/L as against higher levels of 2,4-D.

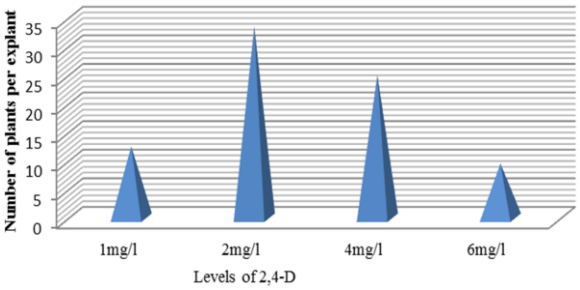

Fig. 2: Plant regeneration in sorghum as influenced by 2,4-D levels

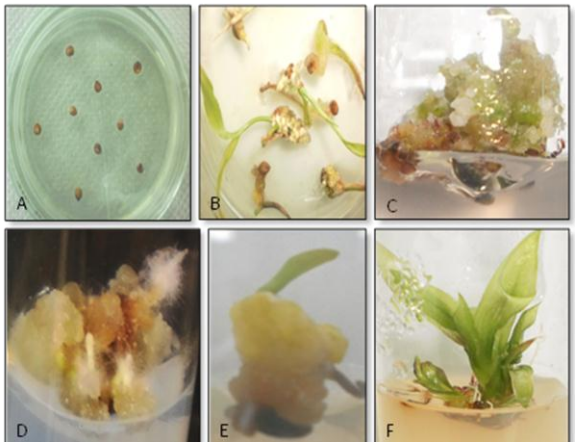

Plate 1: *In vitro* callus-to-plant regeneration from seeds of sorghum

(A). Mature seeds of sorghum line Sandal Bar, B-E) Callus initiation and proliferation from seeds of PC-1 on MS + 2, 4-D (4 mg/L), F) Shoot induction from seed-derived calli of Sandal Bar

We propose that a combination of auxins with cytokinins enhanced the embryogenic callus formation. Both the sorghum lines produced creamy, yellow, compact and higher quantity of callus on 2,4-D- and kinetin-supplemented medium. Other scientists also reported the production of embryogenic callus on 2, 4-D + kinetine medium (Bhaskaran *et al.*, 1987; Kishore *et al.*, 2006; Zhao *et al.*, 2010). However, contrary to the suggestion of Gupta *et al*. (2006), we did not observe any genotype-independent regeneration in the presence of 2,4-D and kinetin-supplemented culture medium. Our study indicated that sorghum callus induction and plant regeneration was genotype- and explant-dependent, this fact is backed by earlier reports (Devi and Sticklen, 2001; Arulselvi and Krishnaveni, 2009). The production of two distinct classes of calli has been frequently reported in cereals and especially in sorghum by Zhao *et al*. (2010). Our findings are also in line with these earlier reports, we identified the embryogenic, non-embryogenic, watery and rhyzogenic types of sorghum calli in our study.

Present study indicated the presence of somaclonal variation in the *in vitro*-raised sorghum plants for various morphological traits, when compared to their parents. Previous reports also confirmed that sorghum somaclones exhibited somaclonal variations for different traits in the preliminary evaluations (Jayaramachandran, 2012; Wambua, 2012).

In conclusion, a highly reproducible tissue culture system has been established for sorghum. The study indicated sorghum line PC-1 as the best *in vitro*-responsive Pakistani sorghum, which has the potential for future

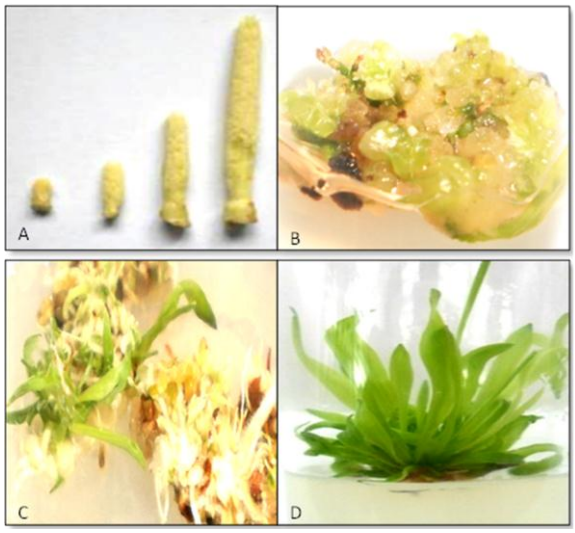

Plate 2: *In vitro* callus-to-plant regeneration from immature inflorescence of sorghum

(A). Immature inflorescences of Sandal Bar, B-C) Callus initiation and proliferation and D) Shoot induction from immature inflorescence-derived calli of PC-1

Plate 3: Sorghum somaclones from lab to field

(A-C) Preparation and transfer of *in vitro* shoots of PC-1 to rooting media, D) Acclimatization of fully developed plants of Sandal Bar in pots, E-F) PC-1 somaclones one month after transfer to field, F) Three months old somaclones of PC-1 in the field

exploitation in somatic hybridization and transformation approaches to create better sorghum.

References

Akram, Z., M.M. Khan, G. Shabbir and F. Nasir, 2011. Assessment of genetic variability in sorghum genotypes for drought tolerance basedon RAPD analysis. *J. Agric. Res*., 49: 455–464

Anbumalarmathi, J. and N. Nadarajan, 2007. Callus induction and plant regeneration in sorghum. *Ind. J. Agric. Res*., 41: 10–16

Arulselvi, I. and S. Krishnaveni, 2009. Effect of hormones, explants and genotypes *in vitro* culturing of sorghum. *J. Biochem. Technol*., 1: 96–103

Belton, P.S. and J.R.N. Taylor, 2004. Sorghum and millets: protein sources for Africa. *Trends Food Sci. Technol*., 15: 94–98

Bhaskaran, S. and R.J. Smith, 1988. Enhanced somatic embryogenesis in *Sorghum bicolor*from shoot tip culture. *In vitro Cell. Dev. Biol*., 24: 65–70

Devi, P.B. and M.B. Sticklen, 2001. Culturing shoot tip clumps of *Sorghum bicolor* and optimal microprojectile bombardment parameters for transient expression. *J. Cytol. Genet*., 2: 89–96

Durrishahwar, N.M., H. Ur-Rahman, Ihteramullah, I.A. Shah, F. Ali, S.M. Ali and N. Mehmood, 2012. characterization of sorghum germplasm for various morphological and fodder yield parameters. *Afr. J. Biotech*., 11: 11952–11959

FAO (Food and Agricultural Organization), 2010. FAOSTAT. http://faostat.fao.org/faostat

Gupta, S., V.K. Khanna, R. Singh and G.K. Garge, 2006. Strategies for overcoming genotypic limitations of *in vitro* regeneration and determination of genetic components of variability of plant regeneration traits in sorghum. *Plant Cell Tiss. Org. Cult*., 86: 379–388

Iqbal, A., B. Sadia, A.I. Khan, F.S. Awan, R.A. Kainth and H.A. Sadaqat, 2010. Biodiversity in the sorghum (*Sorghum bicolor*) germplasm of Pakistan. *Genet. Mol. Res*., 9: 756–764

Jayaramachandran, M., N. Kumaravadivel, G. Kandasamy and Susan Eapen, 2012. Evaluation of somaclonal variations for quantitative traits in fodder sorghum. *Int. J. Bio-res. Stress Manag*., 3: 22–25

Jogeswar, G., D. Ranadheer, V. Anjaiah and P.B.K. Kishor, 2006. High frequency somatic embryogenesis and regeneration in different genotypes of *Sorghum bicolor* from immature inflorescence explants. *In vitro Cell Dev. Biol.-Plant*., 43: 159–166

Kishore, S.N., K.B.R.S. Visarada, A.Y. Lakshmi, E. Pashupatinath, S.V. Rao and N. Seetharama, 2006. *In vitro* culture methods in sorghum with shoot tips as the explants material. *Plant Cell Rep*., 25: 174–182

Linsmaier, E.M. and F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.*, 18: 100-127

Liu, G. and I.A. Godwin, 2012. Highly efficient sorghum transformation. *Plant Cell Rep*., 31: 999–1007

Mani, N.S. and S.R. Pola, 2003. Multiple shoot induction from immature inflorescence in Sorghum. *Cytologia*., 68: 199–204

Manjula, S., Maralappanavar, M.S. Kuruvinashetti and C.C Harti, 2000. Regeneration establishment and evolution of somaclones in *Sorghum bicolor*. *Biol. Med. Life Sci*., 115: 173–180

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*., 15: 473-497

Ngara, R., R. Ndimba, J. Borch-Jensen, O.N. Jensen and B. Ndimba, 2012. Identification and profiling of salinity stress-responsive proteins in Sorghum bicolour seedlings. *J. Proteomics*, 75: 4139-4150

Paterson, A.H., J.E. Bowers, R. Bruggmann, I. Dubchak., J. Grimwood, H. Gundlach, G. Haberer, U. Hellsten, T. Mitros, A. Poliakov, 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature*, 457: 551–556

Pola, S., 2011. Leaf Discs as a Source Material for Plant Tissue Culture Studies of *Sorghum bicolor*(L.) Moench. *Not. Sci. Biol*., 3: 70–78

Prasad, S.R.R., 2012. Development of a transformation system for sorghum (*Sorghum bicolor* L.). *Ph. D. Thesis*, Queensland University of Technology, Australia

Sadia, B., P.C. Josekutty, S.D. Potlakayala, P. Patel, S. Goldman and S.V. Rudrabhatla, 2010. An efficient protocol for culturing meristems of sorghum hybrids. *YTON*, 79: 177–181

Wambua, J.M., 2012. Screening of sorghum (*Sorghum bicolor* L. Moench) somaclonal variants and X-ray radiated seed for salinity tolerance for growth in the Asals. *M.Sc. Dissertaion*. The Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya

Zhao, L.M., S.J. Liu and S.Q. Song, 2008. Efficient induction of callus and plant regeneration from seeds and mature embryos of sweet sorghum. *Chin. Bull. Bot*., 25: 465–468

Zhao, L., L. Shujun and S. Songquan, 2010. Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (Sorghum bicolor). *Afr. J. Biotech*., 9: 2367–2374

(Received 20 October 2012; Accepted 13 March 2013)