
Enhanced shoot regeneration in tissue culture studies of *Sorghum bicolor*

Sudhakar Pola^{1*}, N. Saradamani² and T. Ramana¹

¹Department of Biotechnology, Andhra University, Visakhapatnam 530 003, India.

²Department of Botany, Andhra University, Visakhapatnam 530 003, India.

Pola, S., Saradamani, N. and Ramana, T. (2007). Enhanced shoot regeneration in tissue culture studies of *Sorghum bicolor*. Journal of Agricultural Technology 3(2): 275-286.

The effect of combination of benzyl aminopurine (BAP), thidiazuron (TDZ) and indole acetic acid (IAA) was studied on *in vitro* shoot proliferation from immature embryo explants of *Sorghum bicolor* (L.) Moench, an economically important cereal. Proliferation of multiple shoots was achieved on MS medium supplemented with 1.5 mgL⁻¹ BAP + 1.5 mgL⁻¹ TDZ + 1.0 mgL⁻¹ IAA + 1000 mgL⁻¹ L-asparagine, L-proline, L-glutamine and serine. Upto 72 plantlets per explant were obtained in the present study. Response of six varieties; IS 3566, SPV 475, CSV 13, CSV 15, CSV 112 and IS 348 to multiple shoot formation was studied. The maximum number of shoots were observed in the variety IS 3566. The *in vitro* proliferated and elongated shoots were transferred individually to a root induction medium containing 2% sucrose + 1.0 mgL⁻¹ NAA and within 21 days 162 roots per culture which were produced from multiple shoots. The regenerated plantlets were transferred to 1:1 soil and vermiculite mixture and acclimatized with 60 % survival rate. Fully acclimatized plants were grown in garden soil in greenhouse and their morphological and physiological parameters as similar with seedlings.

Key words: embryogenic callus, immature embryo, multiple shoots, plantlets, *Sorghum bicolor*, thidiazuron.

Introduction

The use of tissue culture as a tool in genetic improvement of most of the cereals has been hampered by the fact that, plant regeneration from various explants is of low frequency and of short duration. Efficient *in vitro* techniques suitable for incorporation of genes and recovery of high number of plantlets are prerequisite for application of transformation technology in crop improvement. In general, callus derived from monocots is more difficult to regenerate *in vitro* when compared with that from dicots (Bahieldin et al., 2000). *Sorghum* is a monocotyledon, a member of Poaceae member has been considered one of the difficult plant species to manipulate through tissue culture (Manjula et al., 2000, Hagio 2002, Harshavardhan et al., 2002, Jeoung

* Corresponding author: Sudhakar Pola; Email: sudhakarpol@gmail.com

et al., 2002, Chandrakanth *et al.*, 2002, Visarada *et al.*, 2003, Kishore *et al.*, 2006, Gupta *et al.*, 2006, Maheshwari *et al.*, 2006) and transformation (Gao *et al.*, 2005, Shrawat and Lörz 2006).

Previous reports by Kresovich *et al.*, (1986) mature embryo; Kuruvinashetti *et al.*, (1998), George and Eapen (1989) immature inflorescence; Sairam *et al.*, (1999) mesophyll derived protoplasts; Zhong *et al.*, (1998), Harshavardhan *et al.*, (2002) shoot apices explants reported multiple shoots from *Sorghum*; but it was less than 10 shoots in a cultures. Efficient regeneration in *Sorghum* was also reported by Mishra and Khurana (2003), Visarada *et al.*, (2003), Nirwan and Kothari (2004), Pola and Mani (2006) Kishore *et al.*, (2006) and Baskaran *et al.*, (2006). However, the rate of plant regeneration per explant is not sufficiently high to be practical application. Therefore a reproducible protocol for efficient plant regeneration from established callus or cell cultures is needed in *Sorghum*. A series of trails were conducted in the present study for enhanced shoot regeneration from immature embryo explants, in view of their importance in crop improvement programmes.

Materials and methods

Surface sterilization of the explant

Immature embryo size ranging from 0.5 to 2.0 mm in length, were isolated/collected from main spikes and used as a source material for embryogenic callus initiation. At first the spikelets were washed with running tap water, surface sterilized with 70 % (v/v) ethanol for one minute and sterilized for 15 min in a 2.5 % (m/v) sodium hypochlorite solution and rinsed with 0.1% HgCl₂ for two minutes, before being meticulously rinsed with sterile distill water. Approximately 50 immature embryos were aseptically removed from the spikelets, using a dissecting microscope and placed with their axes in contact with the callus induction medium and their scutellum positioned upwards on medium containing different concentrations and combinations of auxins and cytokinins.

Preparation of media for callus induction

The media contained MS (Murashige and Skoog, 1962) basal mineral nutrients plus sucrose (3%) was used as media for culture growth. For callus initiation the basal MS media were manipulated with different auxins and cytokinins viz., 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T, IBA, IAA (Indole-3-acetic acid), NAA (α -naphthaleneacetic acid), KN (Kinetin), and ZN

(Zeatin) in different concentrations and combinations from 0.2 mgL^{-1} to 3.0 mgL^{-1} has been used for all experiments. The pH of the media were adjusted to 5.8 using 1N HCl and 1N NaOH. For the preparation of semi solid media, 0.8% agar was used as the gelling agent and the media were heated to boiling for proper mixing. Later, the media were dispensed into appropriate culture vessels i.e., culture tubes, Petri dishes and Baby jars (Borosil, India). The culture vessels were closed with non absorbent cotton, Para film and The Baby jar bottles were capped with polypropylene closures. The sterilization of media and glassware was carried on in an autoclave at $120 \text{ }^\circ\text{C}$ and 15 lb/in^2 pressure for 15 minutes. The chemicals used were of analytical grade (Himedia and Sigma).

Culture maintenance

Cultures were maintained in dark for callus initiation and proliferation at $25\pm 2^\circ\text{C}$ and subculturing was carried out at an interval of 21 days. At the end of first subculture, the cultures containing smooth, shiny globular structures were defined as embryogenic calli and those having unorganized, creamy or yellow coloured structures were recorded as non embryogenic calli. For shoot regeneration, embryogenic calli were detached from the explants and cut into small pieces and these calli were transferred on to regeneration medium containing $0.5\text{--}3.0 \text{ mgL}^{-1}$ of BAP (6-benzylaminopurine)/ZN (Zeatin)/KN (Kinetin) or TDZ (Thidiazuron) medium. The regenerated cultures were incubated at $25\pm 2^\circ\text{C}$ under 16/8 hours (light/dark) photoperiod with cool white fluorescent light giving a photon flux density of $25 \mu\text{mol m}^{-2} \text{ S}^{-1}$ and relative humidity of 50-60%.

Root induction

Regenerated shoots were separated from the cultures individually and used for root induction. The media used for root induction was half strength MS media supplemented with various concentrations of NAA viz., 0.2, 0.5, 1.0 and 1.5 mgL^{-1} .

Establishment of regenerants in the green house

The plantlets, regenerated through various *in vitro* techniques in this study, were taken out from the culture medium and washed gently with double distill water for removing all traces of medium from the roots. The washed plantlets were then transferred to small plastic cups containing sterile sand. The plastic cups were covered with sealed plastic vinyl bags to keep full humidity at $25\pm 2 \text{ }^\circ\text{C}$ in light conditions (photon flux density at $25 \mu \text{ mol m}^{-2} \text{ S}^{-1}$, 16 h).

The plantlets were moistened with water. As the plants grew vigorous, the bags were poked with chopsticks to allow air into the bags until the plants self-supported. The polythene bags were removed after fifteen to twenty days. The plantlets were later transferred to larger pots containing sterile sand and soil (1:1 ratio) and kept under shade in the green house for another two weeks before transferring to field. Fully established regenerants were later established in the field for further growth.

Results and discussion

Callus initiation

For callus initiation different plant growth regulators (P.G.R) at 0.2 mgL⁻¹ to 3.0 mgL⁻¹ concentration was used. Differential callusing ability was observed at different concentrations with maximum production was observed at 2.0 mgL⁻¹ 2,4-D + 0.5 mgL⁻¹ KN in the all the varieties (Pola, 2005) as a result. Further studies were confined to this concentration alone to initiate callus. Explants cultured on 2,4-D + KN medium displayed callus development directly from the scutellum in nearly all cultures on MS nutrient formulations within nine days after inoculation. On 12th day onwards callus grows enthusiastically in dark conditions and from 2nd week onwards. It could discriminate two types of calli based on their colour and compactness. Hard, nodular, compact and white coloured callus referred as embryogenic callus (E.C.), another one is soft, friable, yellowish and mucilaginous cauls referred as non embryogenic callus. In contrast, on growth regulator free medium, the explants neither germinated nor showed any morphogenic response up to 2 weeks.

Shoot regeneration

Both the compact and friable calli were transferred onto the regeneration medium but only the compact calli bestowed regeneration. The white embryogenic callus converted into green colour shoot buds after transferred onto regeneration medium containing different P.G.R s at 1.0 to 3.0 mgL⁻¹ in presence of light (Fig. 2A). Maximum shoot response was observed at 2.0 mgL⁻¹ BAP. In regeneration medium, somatic embryos were gradually changed from white to green colour shoot buds. Then shoot buds were proliferating to shape shoots within 6-12 days (Fig. 2B). The amount of cytokinin used for plant regeneration appeared to be critical in producing vigorous number of shoots. At the outset for regeneration diverse concentrations of BAP, KN, ZN, TDZ and GA3 were used (1.0 to 3.0 mgL⁻¹), but shoot formation was optimum

at 2 mgL^{-1} BAP. Variety IS 3566 and SPV 475 was more responsive at 2 mgL^{-1} of BAP i.e. 18.13 and 16.82 (average) number of shoots per each explant. In CSV 13 maximum shoots are 15.5. While in CSV 15, CSV 112 and IS 348 were respectively 10.52, 9.94 and 9.1. Shoot regeneration can be further enhanced by exposing the organized callus to combination of cytokinins along with different amino acids.

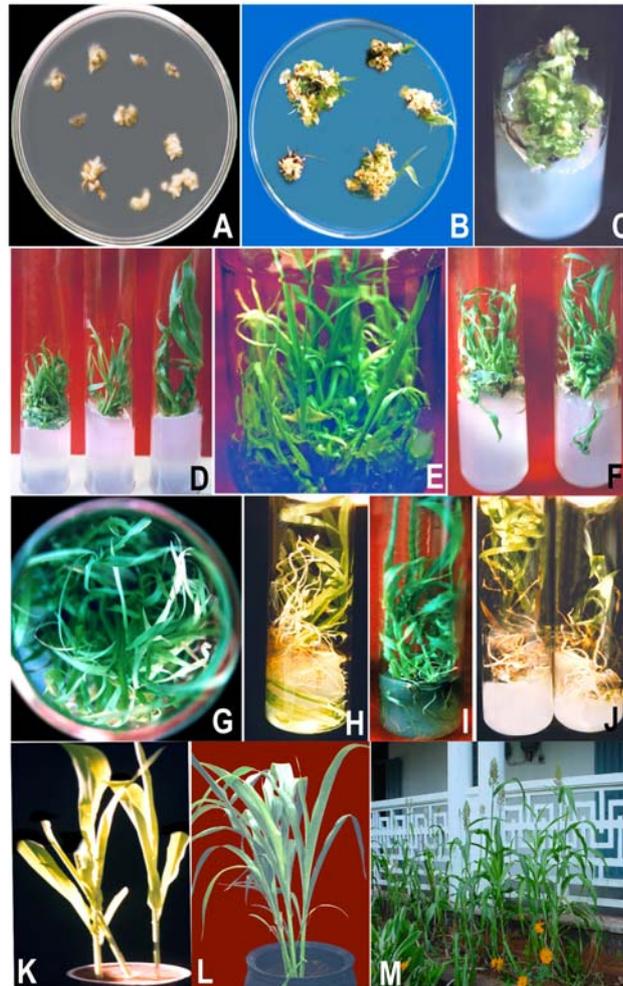


Fig 2. A. Embryogenic callus on regeneration medium. B. Shoot initiation on MS + 2 mgL^{-1} BAP. C. Multiple shoot induction on MS + 1.5 mgL^{-1} BAP + TDZ + 1.0 mgL^{-1} IAA. D.-E. Multiple shoot development on medium with BAP, IAA and TDZ. G. Multiple shoots in a baby jar (upper view). H.-J. Root initiation and development on NAA medium. K. Plantlet acclimatization in earthenware pots. L. Completely established plantlets in green house. M. Completely established plantlets growing on normal conditions.

Combination of cytokinins with auxins

When medium contained BAP only, the plantlets ranged from 9-20 in a single culture in IS 3566, 10-19 in SPV 475, 6-17 in CSV 13, 6-12 in CSV 15, 7-12 in CSV 112 and 4-10 in IS 348, but combination of BAP, TDZ and IAA with addition of 1000 mgL⁻¹ L-proline and L-asparagine produced additional number of shoots (upto 72 shoots per culture (Fig. 3). Addition of BAP and TDZ with IAA at 1.0 mgL⁻¹ enhances multiple shoot formation. Here the combination of BAP and TDZ considerably stimulated the multiple shoot initiation in the presence of IAA (Fig. 2 D). The number of shoots formed per each explant or the production efficiency of multiple shoots varied with the genotypes as well as different concentrations of cytokinins. Maximum number of multiple shoots were observed at 1.5 mgL⁻¹ BAP plus 1.5 mgL⁻¹ TDZ with 1.0 mgL⁻¹ of IAA (72.78 per culture in IS 3566) (Fig. 2E and G). Shoot number was increased by means of steady increase of BAP concentration from 0.5 to 1.5 mgL⁻¹ concentration in the regeneration medium, after 1.5 mgL⁻¹ the shoot number was decreased. The mean number of multiple shoots regenerated from immature embryo is given in the Fig.3. Presence of BAP, TDZ and IAA in the regeneration medium has encouraging influence on plant regeneration. In embryogenesis, these combinations stimulate the production of axillary buds from the embryogenic callus (Fig. 2C) that lead to the formation of multiple shoots. Combination of cytokinins with auxins also proved to be effective for multiple shoot formation in Sorghum. Sairam *et al.*, (1999) reported 8 shoots per explant from mesophyll derived protoplasts of Sorghum using 0.2 mgL⁻¹ KN + 2 mgL⁻¹ BAP. Harshavardan *et al.*, (2002) reported 35-40 shoot buds from the isolated shoot apices using MS+ 5 µM of TDZ + 17.72 µM BAP + 1.074 µM NAA. Nirwan and Kothari (2004) reported multiple shoot induction, using 2 mgL⁻¹ BAP + 0.5 mgL⁻¹ IAA. Recently, Baskaran *et al.*, (2006) reported multiple shoot induction in Sorghum using 13.3 µM BAP + 2,4-D 2.3 µM. Kishore *et al.*, (2006) reported multiple shoots by manipulation of 2.0 mgL⁻¹ BAP, 0.5 mgL⁻¹ 2,4-D and 0.5 mgL⁻¹ TDZ. In monocotyledons species, several authors reported that TDZ induces multiple shoot formation. Shan *et al.*, (2000) demonstrated that TDZ is capable of promoting callus regeneration and it has potential for enhancing the regeneration of cereal and grass species. Gupta and Conger (1998) observed in vitro differentiation of multiple shoot clumps from intact seedlings in switch grass when TDZ was used together with 2,4-D. Aparna and Rashid (2004) reported multiple shoots, with 10 µM TDZ. In the present study also TDZ has been shown to induce multiple shoot formation from embryogenic callus. By means of these reports, use of TDZ and the level of cytokinins and combination with auxins used for

plant regeneration appeared to be critical in producing multiple shoots in Sorghum. In the present study also, combination of 1.5 mgL^{-1} of BAP, TDZ with 1.0 mgL^{-1} IAA bestow enhanced multiple shoot production.

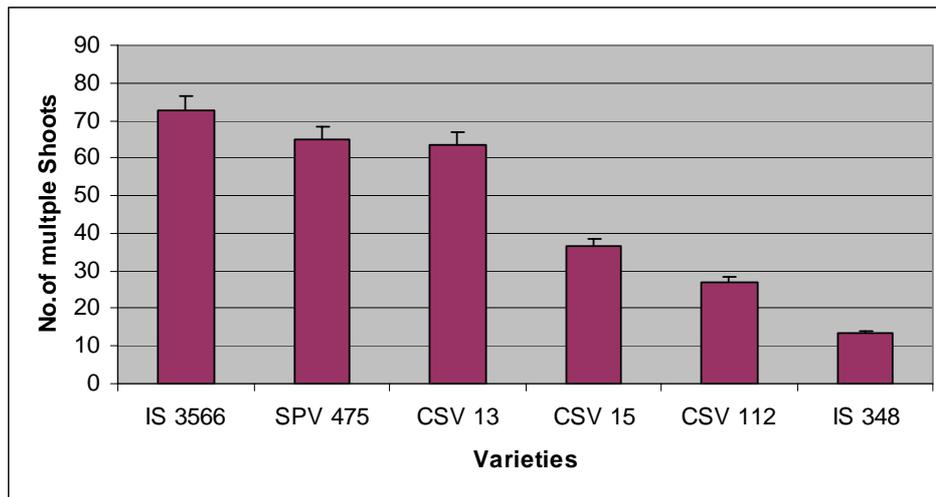


Fig. 3. Average number of Multiple shoots in different varieties.

Effect of photoperiod on cultures

In the present study, results have shown that dark/light conditions are also effective for callus induction and shoot regeneration. Darkness was generally observed to stimulate more somatic embryogenesis than light conditions when auxin is presented in callus induction medium. Experiments on the effect of 16/8 h (light/dark) photoperiod on calli induction and subsequent shoot differentiation were undertaken in the present study. Results indicated that the quality of calli was better, the frequencies of callus induction and subsequent differentiation were higher when callus was induced in total darkness rather than in a 16/ 8 h photoperiod (Fig.1). Phenolic secretions were observed, when callus cultures incubated under light. For regeneration it is entirely reversed. In total darkness the regeneration frequency was poor. When cultures incubated in 16/ 8 h photoperiod 100% shoot regeneration response was observed. Bi *et al.*, (2007) reported that many external factors affect plant growth and development but among them light is the most important because it regulates the whole process of growth and development. Nhut *et al.*, (2000) reported that, in organogenesis the sequence of dark or light affects the rate of differentiation into shoots, and is dependent on the length of exposure of callus culture to the dark. Light inhibited somatic embryogenic formation in plants

such as barley (Kott and Kasha 1984). The effect of light can be interpreted as acting on metabolism and sugar uptake. Furthermore, light may act on energy dependent sugar uptake and metabolism (Nhut *et al.*, 2000). Maheswari *et al.*, (2006) reported that maintaining the cultures in the dark was also absolutely essential for retardation of shoot growth in the callus induction phase. The present results are also agreed with these reports. Thus, dark/light conditions are important factors for callus induction and regeneration response in Sorghum tissue culture.

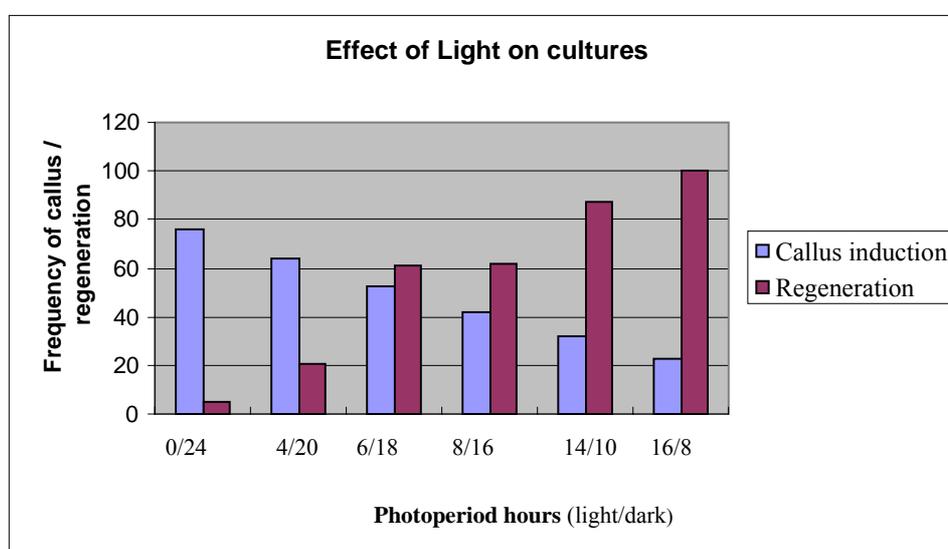


Fig. 1. Effect of Light on callus and regeneration cultures in Sorghum

Effect of amino acids on regeneration

Addition of L-asparagine, L-proline or L-glutamine and serine (1000 mgL^{-1}) to culture medium has enhanced the embryogenic callus formation and regeneration in Sorghum. One reason for the requirement of reduced nitrogen in embryo induction may be that very young embryos lack nitrate reductase, which reduces nitrate to nitrite (Monnier 1990). Organic nitrogen corresponds to a readily incorporated and vigorously economical nitrogen source that may be significant in supporting the growth of cells in culture at times when their nitrate and ammonium assimilative mechanisms are not fully functioning. The concept of addition of amino acids is not a new technique in cereal tissue culture. Previous reports by Armstrong and Green (1985), El’Konin *et al.*, (1995), Rao *et al.*, (1995), Hagio (2002), Vikrant and Rashid (2002), O’Kennedy *et al.*, (2004) obtained enhanced regeneration with the addition of

amino acids in the culture medium. They reported that, the addition of L-proline in tissue culture media might benefit cultured plant tissue; it has a unique function in osmotolerance and morphogenesis as a major constituent of cell wall structural proteins. Furthermore, L-proline may lead to enhance tolerance to stress caused by the tissue culture process (O’Kennedy *et al.*, 2004). This can potentially lead to higher numbers of shoots. The present research showed that addition of 1000 mgL⁻¹ of L-asparagine, L-proline or L-glutamine enhanced shoot formation through somatic embryogenesis. But addition of more than 1000 mgL⁻¹, resulted in tissue necrosis and effects the shoot formation.

Table 1. Effect of different combinations of plant growth regulators on enhanced shoot regeneration from Sorghum immature embryos[#].

Concentration of PGR in mgL ⁻¹			Shoot number	Root number*
BAP	TDZ	IAA		
0.5	0.5	-	33.8	67.9
1.0	0.5	-	42.66	86
1.5	0.5	-	59.8	121.7
0.5	1.0	-	31.2	63
1.0	1.0	-	37	80.9
1.5	1.0	-	41.8	83
0.5	1.5	-	29.82	62.82
1.0	1.5	-	32.78	65.76
1.5	1.5	-	37.51	79.43
0.5	-	0.5	17.55	36.23
1.0	-	0.5	24.77	48.95
1.5	-	0.5	30.66	64.9
0.5	-	1.0	-	-
1.0	-	1.0	16.72	34.5
1.5	-	1.0	22.39	47.1
0.5	-	1.5	-	-
1.0	-	1.5	-	-
1.5	-	1.5	21.6	44.28
0.5	0.5	0.5	25.75	52.94
1.0	1.0	0.5	39.65	82.71
1.5	1.5	0.5	52.82	110
0.5	1.0	1.0	47.45	109.46
1.0	1.0	1.0	61.53	135.67
1.5	1.5	1.0	72.78	162.98
-	0.5	0.5	16.43	36.2
-	1.0	0.5	20.85	22.4
-	1.5	0.5	18.42	36.82
-	0.5	1.0	18.2	36.83
-	1.0	1.0	16.8	33.84
-	1.5	1.0	14.63	29.4
-	0.5	1.5	18.73	35.84
-	1.0	1.5	16.49	32.9

[#]25 explants used for each experiment.

*Rooting medium 1.0 mgL⁻¹NAA.

Rooting

When the plantlets were 3cm long, they were transferred to the rooting medium containing 1.0 mgL⁻¹ NAA (Figure 2H). For rooting, initially we have tested with different auxins (2,4-D, IAA, IBA and NAA) at different concentrations but we observed most excellent performance with 1.0 mgL⁻¹ NAA only. In addition low level of sucrose in the medium and ½ strength MS medium was found to favourable for rooting (Figures 2I and 2J). Shoots after transferring onto the rooting medium, root initiation started within 5 to 8 days and it was depended on shoot number. A maximum of 162.98 roots were observed in IS 3566 (Table 1). Our previous reports in leaf culture (Pola and Mani 2006) also supported NAA for rooting; other reports in Sorghum by Kishore *et al.*, (2006), Maheswari *et al.*, (2006) also supported that 1 mgL⁻¹ NAA is outstanding for rooting.

Establishment of regenerants in the green house

When the regenerated plantlets accomplish well developed root system, were taken out from the culture medium and washed gently with double distil water for removing all traces of medium from the roots and acclimatized as mentioned in the materials and methods. After acclimatization, the plantlets were later transferred to larger pots (Figures 2 K and 2L) containing sterile soil and kept under shade in the green house for another two weeks before transferring to the field. Fully established regenerants were later established in the field for further growth (Figures 2M).

In *Sorghum*, Krersovich *et al.*, (1986) reported multiple shoots, but it was less than 10 only. Visarada *et al.*, (2003) reported 18.7 multiple shoots per explant, when shoot apex was cultured on 4.0 mgL⁻¹ BAP. Mishra and Khurana (2003) reported multiple shoot induction; they obtained 30 plantlets from a single explant in the genotype 296 B, with 0.1 mgL⁻¹ BAP. Nirwan and Kothari (2004) reported 39 shoots per explant from mature embryo cultures and 48 shoots per explant from immature embryo cultures. Anju and Anandakumar (2005) reported 8.64 multiple shoots with 2 mgL⁻¹ BAP from shoot apex as a source explant. Baskaran *et al.*, (2006) reported 35 shoots per callus in *Sorghum*. Whereas, in the present study 20-72 multiple shoots were obtained per single explant. Thus, induction of enhanced shoot regeneration in *Sorghum* for their use in transgenic and genetic engineering experiments can be achieved by selecting suitable source material, culture maintenance, temperature, light and hormonal management.

References

- Anju, V. and Ananadakumar, P. (2005). *In vitro* plant regeneration from different explants of *Sorghum bicolor* (L.) Moench. *Plant Cell Biotechnology And Molecular Biology* 6: 101-108.
- Aparna, G. and Rashid, A. (2004). TDZ-induced somatic embryogenesis in non-responsive caryopses of rice using a short treatment with 2,4-D. *Plant Cell, Tissue and Organ culture* 76: 29–33.
- Armstrong, C. L. and Green, C. E. (1985). Establishment and maintenance of friable, embryogenic maize Callus and the involvement of L-proline. *Planta* 164: 207-214.
- Bahieldin, A., Dyer, W. E., and R. Q.U. (2000). Concentration effect of dicamba on shoot regeneration in wheat. *Plant Breeding* 19:437-439.
- Baskaran, P., Rajeswari, B. R., and Jayabalan, N. (2006). Development of an In Vitro Regeneration System in Sorghum [*Sorghum bicolor* (L) Moench] Using Root Transverse Thin Cell Layers. *Turkish Journal of Botany* 30: 1-9.
- Bi, R., Kou, M. M., Chen, L. G., Mao, S. R., and Wang, H. G. (2007). Plant regeneration through callus initiation from mature embryo of Triticum. *Plant Breeding* 126: 9-12.
- Chandrakanth, E., Sunil, K. G., and Rathore, K. S. (2002). Transgene silencing and reactivation in Sorghum. *Plant science* 162: 181-192.
- El’Konin, L. A., Lopushanskaya, R. F. and Pakhomova, N. V. (1995). Initiation and maintenance of friable, embryogenic callus of *Sorghum bicolor* (L.) Moench by amino acids. *Maydica* 40:153-157.
- Gao, Z., Jayaraj, J., Muthukrishnan, S., Claflin, L., and Liang, G. H. (2005). Efficient genetic transformation of Sorghum using a visual screening marker. *Genome* 48:321-333.
- George, L. and Eapen, S. (1989). Callus growth and plant regeneration in some Indian cultivates of Sorghum. *Current Science* 58: 308-310.
- Gupta, S. D. and Conger, B. V. (1998). In vitro differentiation of multiple shoot clumps from intact seedlings of switch grass. *In Vitro Cellular Developmental Biology of Plant* 34:196–202.
- Gupta, S., Khanna, V. K., Rameshwar, S., and Garg, G. K. (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 Tissue and Organ Culture* 86:379–388.
- Hagio, T. (2002). Adventitious shoot regeneration from immature embryo of Sorghum. *Plant cell, Tissue and organ culture* 68: 65-72.
- Harshavardhan, D., Rani, T. S., Ulaganathan, S. and Seetharama, N. (2002). An improved protocol for regeneration of *Sorghum bicolor* from Isolated Shoot Apices. *Plant Biotechnol* 19(3): 163-171.
- Jeoung, J. M., Krishnaveni, S., Jayaraj, J., Haying, Y., Trick, H.N., Muthukrishna, S., and Liang, G.H. (2002). Agrobacterium mediated transformation of grain Sorghum. In *Sorghum tissue culture and transformation*. Eds. N. Seetharama and I. Godwin. Oxford and IBH Pub. 57-64.
- Kishore, S. N., Visarada, K. B. R. S., Aravinda Lakshmi, Y., Pashupatinath, E., Rao, S. V. and Seetharama, N. (2006). *In vitro* culture methods in *Sorghum* with shoot tips the explant Material. *Plant Cell Reports* 25: 174–182.
- Kott, L. S. and Kasha, K. J. (1984). Initiation and morphological development of somatic embryoids from barley cell cultures. *Canadian Journal of Botany* 62: 1245–1249.
- Kresovich, S., McGee, R. E. and Wadsworth, S. J. (1986). *In vitro* genotypic responses of sweet Sorghum to the synthetic auxins. *Sorghum Newsletter* 29:94-96.

- Kuruvinashetti, M. S., Patil, V. M., Sumangala, B., and Maheshwar, H. (1998). High frequency plant regeneration from embryogenic callus cultures in genus *Sorghum*. *Indian journal of agricultural Sciences* 68(1): 27-28.
- Maheswari, M., Jyothi lakshmi, N., Yadav, S. K., Varalaxmi, Y., Vijaya Lakshmi, A., Vanaja, M. and Venkateswarlu, B. (2006). Efficient plant regeneration from shoot apices of *Sorghum*. *Biologia Plantarum* 50(4): 741-744.
- Manjula, S. M., Kuruvinashetti, M. S. and Harti, C. C. (2000). Regeneration establishment and evaluation of soma clones in *Sorghum bicolor* (L.) Moench. *Euphytica* 115(3): 173-180.
- Mishra, A. and P. Khurana. (2003). Genotype dependent somatic embryogenesis and regeneration from leaf base cultures of *Sorghum bicolor*. *Journal of Plant Biochemistry and Biotechnology* 12: 53-56.
- Monnier, M. (1990). Zygotic embryo culture. In: Bhojwani SS (ed) *Plant tissue culture: applications and limitations*, pp 366-393. Elsevier, Amsterdam.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology* 15: 473-497.
- Nirwan, R. S. and S. L. Kothari. (2004). High frequency shoot organogenesis in *Sorghum bicolor* (L.) Moench. *Journal of Plant Biochemistry and Biotechnology* 13:149-152.
- Nhut, D. T., Bui, V. L., Tran, and Van, K. T. T. (2000). Somatic embryogenesis and direct shoot regeneration of rice (*Oryza sativa* L.) using thin cell layer culture of apical meristematic tissue. *Journal of Plant Physiology* 157: 559-565.
- O’Kennedy, M. A., Smith, G., and Botha, F. C. (2004). Improved regeneration efficiency of a pearl millet (*Pennisetum glaucum* (L) R. Br.) Breeding line. *South Africa journal of Botany* 70(4):502-508.
- Pola, S. (2005). Tissue culture and genetic transformation studies of *Sorghum bicolor* (L.) Moench. Ph.D. Thesis, college of science and technology, Andhra University, Visakhapatnam, INDIA pp 18-28.
- Pola-Sudhakar, R. and N Sarada Mani. (2006). Somatic embryogenesis and plantlet regeneration in *Sorghum bicolor* (L.) Moench, from leaf segments. *Journal of Cell and Molecular Biology* 5(2): 99-107.
- Rao, A. M., Padmasree, K. and Kishor, P. B. K. (1995). Enhanced plant regeneration in grain and sweet Sorghum by asparagine, proline and cefotaxime. *Plant cell reports* 15:72-75.
- Sairam, R. V., Seetharama, N., Devi, P. S., Verma, A., Murty, U. R., and Potrykus, I. (1999). Culture and regeneration of mesophyll derived protoplast of *Sorghum* [*Sorghum bicolor* (L.) Moench]. *Plant Cell Reports* 18: 972-977.
- Shan, X., Li, D. and QU, R. (2000). Thidiazuron promotes in vitro regeneration of wheat and barley. *In vitro cellular Developmental Biology of Plant* 36:207-210.
- Shrawat, A., and Lörz, H., (2006). Agrobacterium-mediated transformation of cereals: a promising approach crossing barriers. *Plant Biotechnology Journal* 4:575-603.
- Vikrant, A., and Rashid, A., (2002). Somatic embryogenesis from immature and mature embryos of a minor millet *Paspalum scrobiculatum* L. *Plant cell, Tissue and organ culture* 69: 71–77.
- Visarada, K. B. R. S., SaiKishore, N., Balakrishna, D., and Rao, S. V. (2003). Transient gus expression studies in *Sorghum* to develop a simple protocol for Agrobacterium mediated genetic transformation. *Journal of Genetics and Breeding* 57:147–154.
- Zhong, H., Wang, W. and Sticklen, M. (1998). *In vitro* Morphogenesis of *Sorghum bicolor* (L) Moench: Efficient plant regeneration from shoot Apices. *Journal of Plant Physiol* 153: 719-726.

(Received 10 June 2007; accepted 30 October 2007)