Vol. 15(4), pp. 546-552, April, 2020
DOI: 10.5897/AJAR2019.13987
Article Number: 13BEE1063497
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Initiation of callus from different genotypes of *Sorghum bicolor* L. Moench

Zeinab Kanani[1]* and Sayadat El Tigani Mohammed

Department of Botany, Faculty of Science, University of Khartoum, P. O. Box 321, Khartoum, Sudan.

Received 28 February, 2019; Accepted 18 July, 2019

The study aimed to examine three genotypes of sorghum for their response in tissue culture using Murashige and Skoog (MS) medium supplemented with auxins (2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA)) and cytokinins (Kinetin, 6-benzyle amino purine (BAP)) at different concentrations. The cultures were initiated from different explants (seed, embryo and hypocotyl). The response of explants varied with the genotype. Callus culture were initiated successfully on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin from G4 (L58) seedling and embryo explants. G2 (94) hypocotyl explants gave callus on media supplemented with 2,4-D only. G5 (2) explants failed to initiate callus but bulging from the embryo explants was observed on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin. The initiated calluses differed morphologically and in their growth rates.

Key words: *Sorghum bicolor,* callus formation, embryo, Kinetin, 2,4-dichlorophenoxy acetic acid (2,4-D).

INTRODUCTION

Successful application of plant biotechnology for plant improvement requires the development of an efficient plant regeneration system from cultured cells or tissues. Many authors have stated that callus derived from monocots is more difficult to regenerate *in vitro* when compared with that from dicots (Bahieldin et al., 2000; Pola et al., 2007).

Sorghum is a monocotyledon, member of Poaceae, it has been considered as one of the difficult plant species to manipulate through tissue culture (Harshavardhan et al., 2002; Kishore et al., 2006; Gupta et al., 2006; Maheswari et al., 2006).

In sorghum, plant regeneration (*via* callus) has been described by many researchers using various explants (Thomas et al., 1977; Ma et al., 1987) such as, immature inflorescences (Elkonin et al., 1995), mature embryo (Kresovich et al., 1986), and mesophyll derived protoplasts (Sairam et al., 1999); also somatic embryogenesis was achieved from shoot tip explants of sorghum (Seetharama et al., 2000). Efficient regeneration in sorghum tissue culture was also reported by many researchers (Mishra and Khurana, 2003; Pola and Mani, 2006; Kishore et al., 2006). However, the rate of plant regeneration per explant is not sufficiently high to be of practical application (Pola et al., 2007).

The main objective of the study was to examine three genotypes of sorghum for their response in tissue culture using Murashige and Skoog medium supplemented with

*Corresponding author. E-mail: zeinab_mohd@yahoo.com.

different auxins and cytokinins at different concentrations

MATERIALS AND METHODS

Three lines of sorghum (*Sorghum bicolor*), namely 94, L58 and 2 referred to by G2, G4 and G5, respectively were used to find out the most suitable genotype for maximum callus production. Seeds of these cultivars were obtained from the Agronomy Department, Faculty of Agriculture, University of Khartoum. Harvests of 2009/2010 were used.

Seeds, embryos, hypocotyls, epicotyls and leaves were chosen as explants for the study. Seeds of three selected genotypes G2, G4 and G5 were surface sterilized with 70% (v/v) ethanol for 1 min and then immersed for 15 min in a 2.5% (v/v) sodium hypochlorite solution or "Clorox" in a presence of few drops of a liquid detergent. Then, the seeds were rinsed 3 to 4 times with sterile distilled water.

Sterile seeds were grown in moistened filter paper as well as in solidified MS basal medium. One-day-old embryos were separated and cultured in MS basal medium supplemented with different components in order to initiate callus, 7-day-old hypocotyls and leaves were selected from the *in vitro* raised seedlings and used as explant for callus initiation.

The media contained MS basal mineral nutrients (Murashige and Skoog, 1962) supplemented with 3% sucrose were used as media for culture growth. For callus initiation the basal MS media were manipulated with auxins {indole- butyric acid (IBA); naphthalene acetic acid (NAA); 2,4- dichlorophenoxy acetic acid (2,4-D)}, and cytokinins {6-benzyle amino purine (BAP), and Kinetin}, in different concentrations and combinations in a range from 1 to 12.5 mg/l have been used in all experiments. L-Ascorbic acid at 100 mg/l was added as an anti-oxidant agent. The pH of the media was adjusted to 5.7. For preparation of semi solid media, 0.8% agar was used as the gelling agent.

The sterilization of media and glassware was carried out in an autoclave at 121°C, (15 lb/in^2) pressure for 15 min before dispensing in culture vessels.

Callus multiplication and maintenance

For callus multiplication and maintenance the MS medium was supplemented with 2 mg/l 2,4-D and 0.5 mg/ml Kinetin in the presence of 3% sucrose, 0.8% agar and 100 mg/l L-ascorbic acid. Also, 2 mg/l 2,4-D was used without Kinetin in the presence of other supplements for the same purpose.

RESULTS AND DISSCUSION

In the preliminary experiment, it was found that three out of five genotypes of *S. bicolor* studied for salinity tolerant G2, G4 and G5 were efficient for callus induction when different explants were used. For callus initiation, healthy explants of mature seeds, embryos and segments of hypocotyls and leaves were used as the source materials.

Callus induction

Sorghum mature embryos from the three genotypes were removed under aseptic conditions and transferred aseptically to MS medium supplemented with 3% sucrose, 2 mg/l 2,4-D, 0.5 mg/l Kinetin and the medium was solidified with 0.8% agar.

After 34 days, G2 cultured embryos failed to show tissue proliferation and failed to form callus (Figure 1a). On the other hand G4 embryos proliferated rapidly forming a prominent callus within 34 days (Figure 1b). G5 embryos bulging appeared in certain parts of the embryo tissue (Figure 1c).

When whole grain of *S. bicolor* from G4 were used for callus initiation on the same medium used for the mature embryo, callus initiation on the surface of seedling was achieved from all seedlings grown under aseptic conditions.

Callus growth and morphology

The growth rate of the initiated callus was followed for a period of 90 days (Figure 2a to e).

Low levels of 2,4-D have been the most commonly used auxin for callus induction in the cereals. Lu et al. (1983), Hagio (1994), Bi et al. (2007) and Pola et al. (2008) reported that cereals in general require 2,4-D to initiate callus culture and its higher concentrations have been found to be less effective in the formation of embryogenic callus.

Pola et al. (2008) reported that 2 mg/l 2,4-D was the optimum concentration to obtain high frequency of embryogenic callus in sorghum.

A similar trend was observed in the present study. These results revealed that a combination of auxins with cytokinin boosted the embryogenic callus formation.

Previous reports by Maheswari et al. (2006), Gupta et al. (2006), Pola and Mani (2006) and Pola et al. (2008) showed that auxin and cytokinin combination will improve embryogenic callus induction. Gupta et al. (2006) suggest that, to overcome the genotypic limitation of plant regeneration from callus in sorghum, the callus induction medium must be supplemented with strong cytokinin like kinetin with 2,4-D.

When whole grains of *S. bicolor* G2 were grown on MS medium supplemented with 1 mg/l NAA plus 2 mg/l BAP, the explant showed dark purple exudation diffused to the medium. However, when that of G4 supplemented with 1 mg/l NAA plus 2 mg/l BAP were used, brown exudate was produced (Figure 3a and b).

G5 seedlings cultured on the same medium gave abnormal seedlings, showing tissue proliferation in the hypocotyl region (Figure 3c). No exudate was released to the medium.

These results agree with those reported with Pola et al. (2008) who reported that callus initiation was accompanied by the exudation of dark brown and purple colour pigment leading to the brown colorization of the medium around their bases and these cultures underwent necrosis.

To control these phenolic secretions, 0.1% L-ascorbic

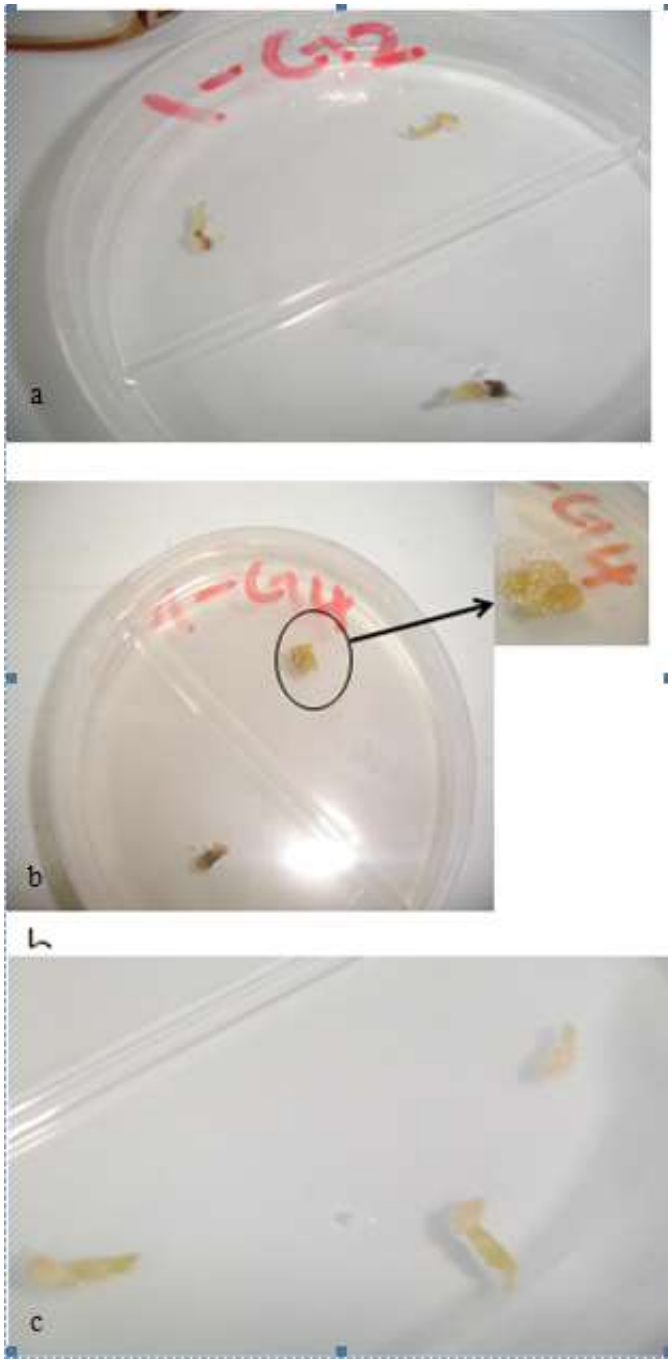

Figure 1. Mature embryo explants grown on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l kinetin for a period of 34 days. (a) G2 no callus initiation (×1), (b) G4 callus initiation (×3/4), (c) G5 bulging for embryo tissue (×1.5).

acid was used in culture media. Frequent subculturing to fresh media with same composition was also tested to overcome the problem.

Segments from seedlings grown under aseptic conditions were also used as explant for callus initiation. Hypocotyl and leaf segments were inoculated to MS medium supplemented with 2 mg/l 2,4-D only. Explants from G2 initiated callus at the cut end of the hypocotyl segments (Figure 4), while leaf explants failed to initiate callus.

The cultures response was greatly influenced by the genotype in all types of explants. Genotypes effects on callusing ability from sorghum were reported previously by Cai and Butler (1990).

Calli formed from all the explants, usually appear in two types based on their colour and quality, that is, friability or compactness. These two types were similar to the embryogenic and non-embryogenic types of callus described earlier in sorghum by Cai and Butler (1990) and more recently by Pola et al. (2008). The embryonic callus appeared yellow, comparatively more compact and morphogenic in nature. At the same time the non-embryogenic callus were unorganized, friable, soft, loosely packed and pale yellow or dull creamy in colour (Figure 2).

General embryogenic callus showing globular structure was visible on the 34th day after inoculation. Changes in the callus morphology were observed in embryogenic and non- embryogenic callus by increasing the number of subcultures. Formation of globular compact or loose friable callus was observed in all callus initiated from the different genotypes, irrespective of their auxin type or concentration. Pola et al. (2008) reported that callus induction frequencies ranged from 40 to 84%. In this study, callus induction frequency was the highest in G4, that is, 100% on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin (Figure 2).

The total callus area was estimated in all types of calluses. Of the three genotypes used in this study, genotypic differences were observed with respect to total callus amount. The total area of the callus was highest in genotype G4 and minimum in G5 (Figure 1).

For the study of growth rate of the callus, daily measurements were recorded for all initiated calluses. In general, maximum growth rate, in term of increase of callus area was observed in G4 (Figure 2).

Therefore, among the three genotypes studies, the most suitable genotype to produce maximum embryogenic callus was that of G4. The G4 genotype, also illustrated higher value in term of periodicity of embryogenic callus, quantity of embryogenic callus and growth rate. While genotypes G2 and G5 showed lower rate for most of the characters. These genotypic differences were also observed by Hagio (1994), Gupta et al. (2006), Jogeswar et al. (2007) and Pola et al. (2008) in sorghum.

In the present study, the use of mature embryo tissue provides high embryogenic callus induction frequency, while mature explant failed to show such efficient response. In fact, this was the most critical factor for obtaining large amount of callus tissues which may lead to the formation of large number of somatic embryos from mature embryos.

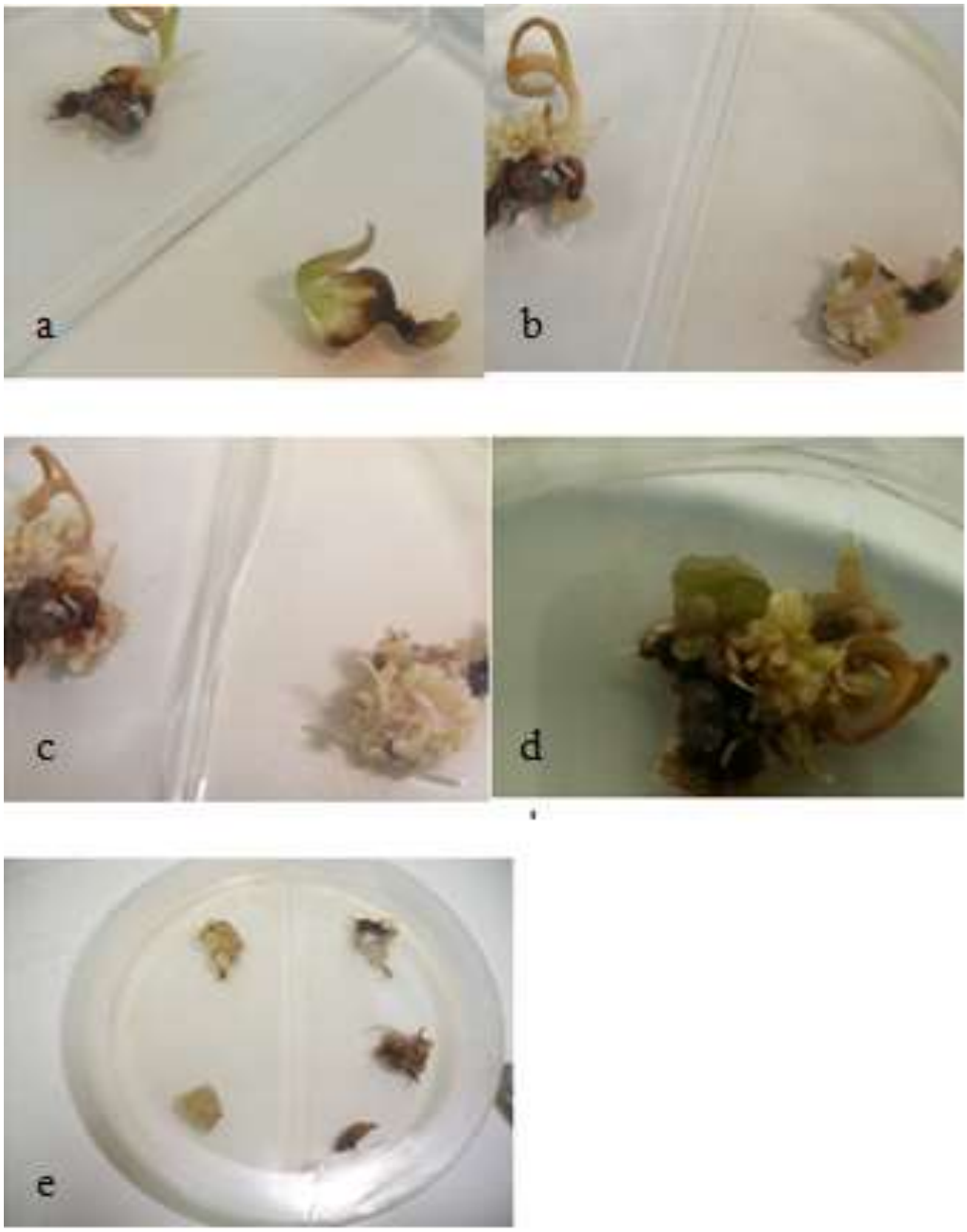

Figure 2. *S. bicolor* G4 callus initiation from sterile seedlings on MS medium with 2 mg/l 2,4-D and 0.5 mg/l kinetin at different stages of development. (a) 27 days after inoculation (×1), (b) 54 days after inoculation (×1), (c) 79 days after inoculation (×1), (d) 84 days after inoculation (×1.5), (e) 90 days after inoculation (×3/4).

Bhojwani and Razdan (1996) reported that, the ability to form large number of somatic embryos from immature embryos is especially true for cereals. Rathus et al. (2004) showed that the physiological stage of source material (explant) used for callus initiation was found to be critical. Gupta et al. (2004) also reported that immature embryos size influenced callus formation and plant regeneration in sorghum.

The callus culture incubated under diffused light underwent necrosis due to the phenolic exudation (Figure 3). These results agree with those reported by Pola et al. (2008).

Recently, many researches are published dealing with *in vitro S. bicolor* tissue culture and regeneration (Pola et

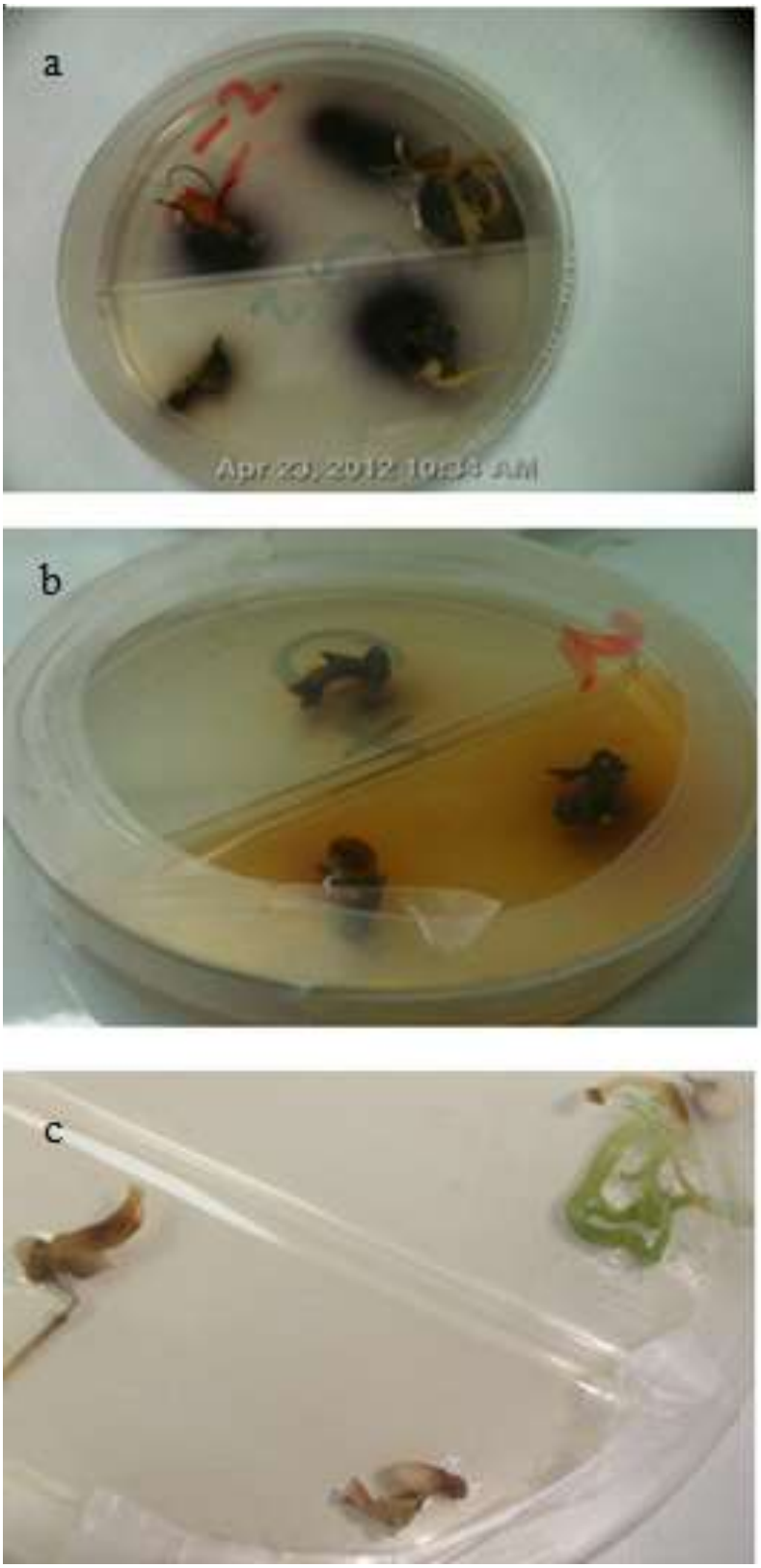

Figure 3. *S. bicolor* seeds grown on MS medium supplemented with 1 mg/l NAA and 2 mg/l BAP. (a) G2, dark purple exudates around the germinating seeds (×1/2). (b) G4 brown exudates on the culture medium (×1). (c) G5 no pigment exudation, growth of seedlings in abnormal and showing tissue proliferation in the hypocotyl region (×1.25).

Figure 4. *S. bicolor* G2 callus initiation from hypocotyl and maintained on MS medium supplemented with 2 mg/l 2,4-D (×2).

al., 2007, 2008).

In most of these studies, they concentrated on the choice of explant and regeneration using various growth regulators.

Conclusion

The study revealed that the amount of the callus formed was genotype and type of explant dependent in the concentration of 2,4-D used. These differences in callusing ability of the different genotypes need further study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERNCES

Bahieldin A, Dyer WE, Qu R (2000). Concentration effect of dicamba on shoot regeneration in wheat. Plant Breeding 19:437-439.

Bhojwani SS, Razdan MK (1996). Plant Tissue Culture Theory and Practice. Elsevier, New York.

Bi R, Kou MM, Chen LG, Mao SR, Wang HG (2007). Plant regeneration through callus initiation from mature embryo of *Triticum.* Plant Breeding 126:9-12.

Cai T, Butler L (1990). Plant regeneration from embryogenic callus initiated from immature inflorescence of several high tannins *Sorghum*. Plant Cell, Tissue and Organ Culture 20:101-110.

Elkonin LA, Lopushanskaya RF, Pakhomova NV (1995). Initiation and maintenance of friable embryogenic callus of sorghum (*Sorghum bicolor* (L.) Moench) by amino acids. *Maydica* 40:153-157.

Gupta S, Khanna VK, Rameshwar S, Garg GK (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.

Gupta S, Khanna VK, Singh R, Garg GK (2004). Identification of *in vitro* responsive immature embryo size for plant regeneration in Sudan grass (*Sorghum sudanense*). Indian Journal of Biotechnology 3:124-127.

Hagio T (1994). Varietal differences of plant regeneration from callus of *Sorghum* mature seed. Breeding Science 44:121-126.

Harshavardhan D, Rani TS, Ulaganathan S, Seetharama N (2002). An improved protocol for regeneration of *Sorghum bicolor* from isolated shoot apices. Plant Biotechnology Journal 19(3):163-171.

Jogeswar G, Ranadheer D, Anjaiah V, Kavikishor PB (2007). High frequency somatic embryogenesis regeneration in different genotypes of *Sorghum bicolor* L. from immature inflorescence explant. *In Vitro* Cell and Developmental Biology 43:159-166.

Kishore SN, Visarada, KBRS, Aravinda Lakshmi Y, Pashupatinath E, Rao SV, Seetharama N (2006). *In vitro* culture methods in *Sorghum* with shoot tips the explant material. Plant Cell Reports 25:174-182.

Kresovich S, McGee RE, Wadsworth SJ (1986). *In vitro* genotypic responses of sweet Sorghum to the synthetic auxins. Sorghum Newsletter 29:94-96.

Lu C, Vasil V, Vasil IK (1983). Improve efficiency of somatic embryogenesis and plant regeneration in tissue culture of maize (*Zea mays* L.). Theoretical and Applied Genetics 66:285-289.

Ma H, Gu M, Liang GH (1987). Plant regeneration from cultured immature embryos of *Sorghum bicolor* (L.) Moench. Theoretical and Applied Genetics 73:389-394.

Maheswari M, Jyothi lakshmi N, Yadav SK, Varalaxmi Y, Vijaya Lakshmi A, Vanaja M, Venkateswarlu B (2006). Efficient plant regeneration from shoot apices of *Sorghum*. Biologia Plantarum 50(4):741-744.

Mishra A, Khurana P (2003). Genotype dependent somatic embryogenesis and regeneration from leaf base cultures of *Sorghum bicolor*. Journal of Plant Biochemistry and Biotechnology 12:53-56.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15(3):472-497.

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

Pola S, Saradamani N, Ramana T (2008). Plant tissue culture studies in *Sorghum bicolor* immature embryo explant as the source material. International Journal of Plant Production 2(1):1-14.

Pola SR, Mani SN (2006). Somatic embryogenesis and plantlet regeneration in *Sorghum bicolor* (L.) Moench, from leaf segments. Journal of Molecular Cell Biology 5(2):99-107.

Rathus G, Nguyen T, Able JA, Gray SJ, Godwin D (2004). Optimizing *Sorghum* transformation technology *via* somatic embryogenesis. In: Seetharama, N. and Godwin, I. (eds.). Sorghum Tissue Culture and Transformation, pp. 25-34.

Sairam RV, Seetharama N, Devi PS, Verma A, Murty UR, Potrykus I (1999). Culture and regeneration of mesophyll derived protoplast of sorghum [*Sorghum bicolor* (L.) Moench]. Plant Cell Reports 18:972-977.

Seetharama N, Sairam RV, Rani TS (2000). Regeneration of sorghum from shoot tip cultures and field performance of the progeny. Plant Cell, Tissue and Organ Culture 61(2):169-173.

Thomas E, King PJ, Portykus I (1977). Shoot and embryo-like structure formation from cultured tissues of *Sorghum bicolor*. Naturwissenschaften 64:587.