Sjemenarstvo 26 (2009) 3-4

UDK: 633.174 (045) = 862
Original scientific paper

MATURE EMBRYO AS A SOURCE MATERIAL FOR EFFICIENT REGENERATION RESPONSE IN SORGHUM (*Sorghum bicolor* L. Moench.)

SUDHAKAR POLA[1], N. SARADAMANI[2] and T. RAMANA[1]

[1]Department of Biotechnology
[2]Department of Botany

Andhra University,
Visakhapatnam, India

SUMMARY

Efficient plant regeneration is a prerequisite for a complete genetic transformation protocol in cereals. Aiming this, in the present study, we have accomplished efficient plant regeneration using mature embryos as a source material in *Sorghum bicolor* (L.) Moench. Although immature inflorescence and immature embryos are best explant sources for *in vitro* culture in *Sorghum*, however they are available only for a limited period in a year. Mature embryos have always been ideal for *in vitro* studies for the reason that they can be handled easily over other explants and available throughout the year.

Mature embryo explants of *Sorghum bicolor* genotypes viz. IS 3566, SPV 475, CSV13, CSV15, CSV112, IS 348 were cultured on MS medium for efficient callus induction and subsequent plant regeneration. The response of different combination and concentrations of plant growth regulators were compared, and factors affecting the mature embryo tissue culture response were studied in this manuscript. Significant genotypic differentiation was detected in embryogenic callus induction and plantlet regeneration. Genotype IS 3566 showed better tissue culture response than the other genotypes. Efficient embryogenic callus induction was achieved with 2mg l^{-1} 2, 4,5-Trichlorophenoxyacetic acid (2,4,5-T) and multiple shoot induction was achieved by manipulation of 6-benzyl adenine (BAP), Thidiazuron (TDZ), and Indole-3-acetic acid (IAA) in the culture medium.

Keywords**:** *Sorghum bicolor,* embryos, 2, 4,5-T, embryogenic callus, BAP, TDZ, regeneration

INTRODUCTION

Sorghum bicolor (L.) Moench is an agriculturally important cereal crop plant. Worldwide annual production of *Sorghum* is about 60 million tones, less than that of the other major cereal crops. It is nonetheless a staple for both humans and livestock, and is also a potential source of biofuel. *Sorghum* originates from tropical Africa, which makes it highly tolerant to drought and well adapted to arid countries in northeast Africa, where it is mainly grown, as well as to dry areas in the United States and India (Sasaki and Antonio 2009). Despite its use as food, feed, fodder, fiber and fuel, there is a rising demand for *Sorghum* towards the production of alcohol, beer and other *Sorghum* based derivatives such as syrup and jaggery (Ratnavati et al., 2003, Kishore et al., 2006). In the developing world, improving *Sorghum* through genetic transformation is the latest in a long series of technologies that have been applied to this crop.

Cereal crop improvement through genetic transformation requires establishment of an efficient and reproducible plant regeneration system (Chang *et al.,* 2003). Efficient plant regeneration is a prerequisite for a complete genetic transformation protocol (Jha et al., 2009). On the other hand *Sorghum* is considered to be the one of the most recalcitrant species among the cereals for *in vitro* response and genetic transformation (Gao et al., 2005, Gupta et al., 2006 and Kishore et al., 2006, Nguyen et al., 2007, Sudhakar et al., 2008). Also, the regeneration of differentiated cereal plant cells from callus remains a major limiting step in obtaining high numbers of cereal clones or independent transgenic cereal lines (Eudes et al. 2003). In view of the above, a series of trails were conducted in the present study for enhanced shoot regeneration from mature embryo explants, in view of their importance in crop improvement programs.

Mature seeds are the most preferred explants for *in vitro* protocols as they can be stored, available round the year and can easily handled (Kishore et al., 2006). Earlier, A limited number of studies has been carried out on *in vitro* culture of mature embryos of *Sorghum*, Bhaskaran et al., 1983, obtained sodium chloride tolerant callus derived from mature embryo. Smith et al., 1983, reported plant regeneration from mature embryos derived callus on aluminum selected media. Waskam et al., (1990) and Miller et al., (1992), also studied *in vitro* culture of mature embryos in mature embryos *Sorghum*. On the other hand, the rate of plant regeneration is not satisfactorily high to be practical relevance. As a result, a reproducible protocol for proficient plant regeneration is desirable in *Sorghum bicolor*.

The aim of the present research was to study the effect of different constraints on shoot regeneration of *sorghum* using mature embryo as the starting material to identify an ideal genotype for plant tissue culture and genetic transformation.

MATERIALS AND METHODS

Seeds of *Sorghum* variety SPV 475, IS 3566, CSV 13, CSV 15, CSV 112 and IS 348 were used as source material. These seeds were surface sterilized in 70% ethanol for 5 min, rinsed twice with sterilized distilled water, incubated further in 50% sodium hypochlorite solution for 25 min, and rinsed 7 times in sterilized distilled water. The surface sterilized seeds were incubated at 25 °C for 2 h in sterilized distilled water for imbibitions. The mature embryos were removed from the imbibed seeds and placed, scutellum-up, on MS medium (Murashige and Skoog 1962) supplemented with 20 mg l^{-1} sucrose, 2 mg l^{-1} 2,4,5-T, 8 g l^{-1} agar and incubated at 25 ± 2 °C for 14 days in darkness (Pola 2005). At the end of this incubation period, the callus tissues were transferred to MS + BAP/TDZ medium for initiating root and shoot development (regeneration) and incubated for 5 weeks at 25 ± 2 °C in a 16 h light (2000 lux) 8 h dark photoperiod. The 1-2 cm tall plantlets that developed roots and shoots were transferred to baby jars containing MS + NAA medium and incubated for a further month. As the roots of these plantlets reached 10-12 cm, they were transferred to pots containing sterilized garden soil. In order to attain high humidity (acclimatization) the pots were covered with plastic film and kept at 25 ± 2 °C in a 16 h light 8 h dark photoperiod. After 3 weeks of maintenance, plantlets were transferred to soil in a greenhouse. For each genotype a completely randomized design with 3 replications (25 embryos per replication) was constructed to collect data on the frequency of embryogenic, number of shoots and roots obtained.

RESULTS AND DISCUSSION

Effect of plant growth regulators on callus induction

The role of plant growth regulators in cereal tissue culture is very important. In the present study, on plant growth regulator free medium, the explants neither germinated nor showed any morphological response up to one week. In contrast, explants cultured on auxin medium exhibited callus formation in almost all cultures, detectable callus formation was obtained within five days and observation was taken at 10th day of the culture. In the second week, two types of calli were observed in the culture. A soft and friable callus that was yellowish and mucilaginous in nature and another one is a hard, nodular, compact and white embryogenic callus. Compact embryogenic calli mainly exhibit differentiation of somatic embryos so further experiments were continued with this type of calli only.

From the 25 growth regulator concentrations tested in this study almost all were suitable for inducing embryogenic callus, on the other hand, the frequency and type of

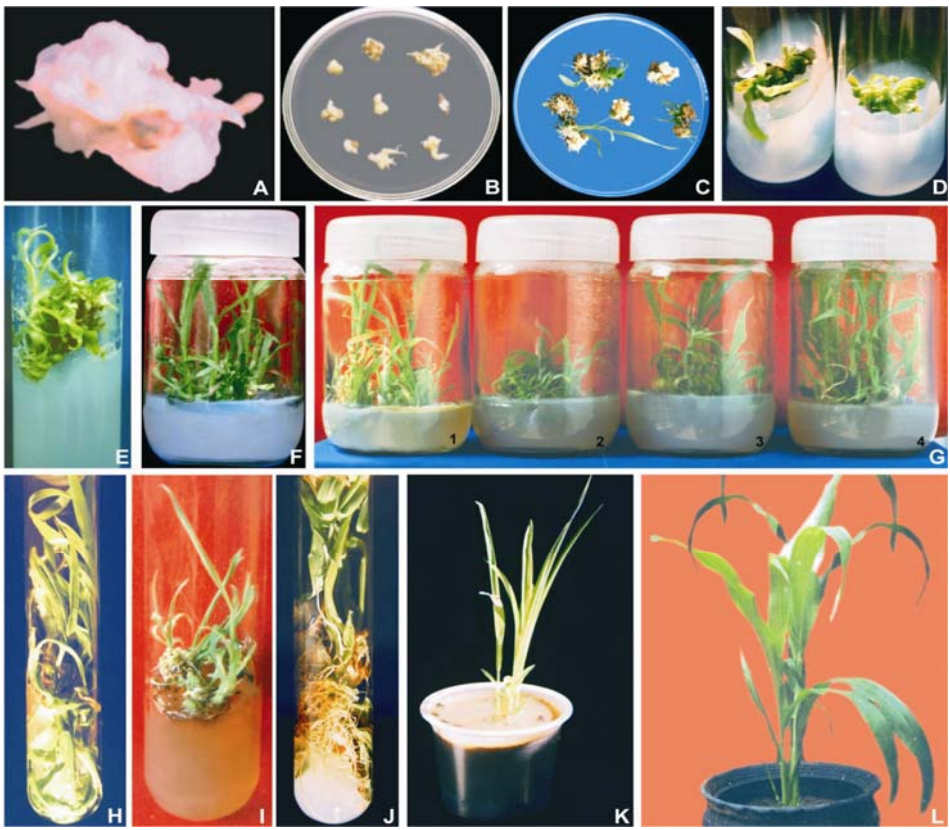
Table1 Effect of PGR on embryogenic callus induction from mature sorghum embryo
Tablica 1. Utjecaj hormona rasta na indukciju embriogentskog kalusa zrelog embrija sirka

Concentration of PGR *Koncentracija hormona rasta* (mg l^{-1})					Embryogenic Callus Frequency* *Frekvencija embriogenetskog kalusa* (%)
2,4-D	2,4,5-T	IBA	IAA	NAA	
1.0					48
1.5					56
2.0					64
2.5					72
3.0					64
	1.0				56
	1.5				72
	2.0				84
	2.5				64
	3.0				56
		1.0			40
		1.5			54
		2.0			60
		2.5			62
		3.0			50
			1.0		40
			1.5		52
			2.0		56
			2.5		56
			3.0		40
				1.0	40
				1.5	56
				2.0	66
				2.5	56
				3.0	48

*Each value is an average number of 3 replications using 25 explants per replication
**Svaka vrijednost prosjek je triju ponavljanja sa po 25 eksplantanta*

PGR: Plant growth hormone
PGR: Hormoni rasta

Figure 1 *In vitro* plant regeneration from mature embryo explants of *Sorghum bicolor*
Slika 1 In vitro regeneracija zrelog eksplantanta embrija sirka



A. Embryogenic callus development on MS + 2 mg l^{-1} 2,4,5-T.
B. Embryogenic callus showing development of somatic embryos on Regeneration medium.
C. Differentiation / Shoot initiation on MS + 1.5 mg l^{-1} BAP
D. Shoot regeneration on MS +1.5 mg l^{-1} TDZ
E. Proliferation of multiple shoots on MS + 1.5 mg l^{-1} TDZ + BAP + IAA medium.
F. Multiple shoots in the genotype of IS 3566.
G. Multiple shoots in the genotype of 1. CSV112, 2. IS 348, 3. CSV 15, 4. SPV 475
H. Six weeks old well developed plantlet in MS + asparagines & proline medium
I. Root initiation on MS + 1 mg l^{-1} NAA
J. Regenerated plantlet having fully developed root and shoot system is ready to transfer to the *ex vitro* condition.
K. Hardened regenerated plants in the Greenhouse.
L. Hardened plant in the filed (30 days after transferring to the filed).

callus varied significantly. Studies in cereals showed that, the use of 2, 4-dichlorophenoxyacetic acid (2,4-D) to induce callus formation from mature embryos was a critical factor. In general, auxins usually 2,4-D in the range of 1–3 mg l^{-1} , is essential for the formation of embryogenic callus from cereal embryos (Bi et al., 2007). Hagio (1994) and Manjula et al., (2000) reported that cereals require low level of 2, 4-D to initiate the callus cultures and its higher concentrations have been found to be less effective in the formation of embryogenic callus. As a result, initially we started experiment with 2, 4-D a similar tendency was observed in the present study also, i.e., 2.5 mg l^{-1} 2, 4-D was optimum concentration to obtain high frequency of embryogenic calli (72%).

Further we extended our experiment with other auxins like IBA (Indole-3-butyric acid), IAA, NAA and 2,4,5-T. The effect of IBA, IAA and NAA **(**1-naphthylacetic acid) are lesser effective than 2,4-D but 2 mg l^{-1} 2,4,5-T in the culture medium was significantly boost the embryogenic callus frequency up to 84% (Table1 & Figure 1A). Very few reports available on addition of 2,4,5-T in the culture medium for callus induction, Pola and Mani (2006) reported somatic embryogenesis from *Sorghum* leaf explants using 2mg l^{-1} 2,4,5-T. Mikhail et al., (2006) reported embryogenesis and plant regeneration from mature embryos of wheat using 2,4,5-T. Jogeswar et al., (2007) used 2,4,5-T in the culture medium for somatic embryogenesis in *Sorghum bicolor*.

Effect of photoperiod

Our initial experiments in *in vitro* culture of immature embryo in *Sorghum* (Pola et al., 2007 & Pola et al., 2008) 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 and 16/8 h photoperiod is suitable for efficient regeneration. As a result, in this study we maintained our mature embryo cultures on total darkness for callus induction and 16/8 h photoperiod for regeneration.

Regeneration

After the embryogenic callus clumps were transferred to the shoot development medium, green shoots developed from somatic embryos (Figure 1B). Spontaneous development of shoots and plantlets from embryogenic callus was observed on cytokine medium only. Media free of BAP and TDZ or supplemented only with auxins were not effective for improved differentiation of shoots. For regeneration different concentrations (0.5, 1.0, 1.5 and 2.0) and combination of BAP, TDZ and IAA were used in the regeneration medium. When these growth regulators used alone, the highest frequency (22.64%) was recorded with TDZ at a concentration of 1.5 mg l^{-1} . BAP gives 20.28% shoot regeneration at a concentration of 1.5mg l^{-1} (Table 2).

Effect of asparagines and proline on regeneration

In our previous study (immature embryo culture, Pola et al., 2007) addition of L-asparagine, L-proline or L-glutamine and serine (1000 mg l^{-1}) to culture medium has enhanced the embryogenic callus formation and regeneration in *Sorghum* (Figure 1 H).

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 O'Kennedy *et al*., (2004) obtained enhanced regeneration with the addition of amino acids in the culture medium. Recently, Asad et al., (2009) reported the efficient regeneration through inclusion of amino acids in regeneration medium of sugarcane. Consequently we continued the addition of L-asparagine, L-proline or L-glutamine and serine in this study also.

Combination of BAP, TDZ with IAA

A remarkable stimulation of multiple shoot formation was observed when BAP and TDZ were combined with an auxin IAA. Despite the fact that, IAA is an auxin, it can induce callus/root initiation only, but when low level of IAA combined with BAP and TDZ that encourage the shoot regeneration. Regeneration medium supplemented only with IAA is not effective for differentiation of shoots and it persuades the initiation of roots directly from the embryogenic callus. When medium contained BAP/TDZ only, the plantlets ranged from 12-18 in a single culture (Figure 1 C, D), but combination of BAP, TDZ and IAA with addition of 1000 mg l^{-1} L-proline and L-asparagine produced additional number of shoots (43.22 shoots per culture (Figure 1.E). Addition of BAP and TDZ with IAA at 1.0 mg l^{-1} enhances multiple shoot formation. Here the combination of BAP and TDZ considerably stimulated the multiple shoot initiation in the presence of IAA (Figure 1 F), the number of shoots formed per each explant or the production efficiency of multiple shoots varied with the genotypes (Figure 1 G), as well as different concentrations of cytokinins. Maximum number of multiple shoots were observed at 1.5 mg l^{-1} BAP plus 1.5 mg l^{-1} TDZ with 1.0 mg l^{-1} of IAA (43.22 per culture in IS 3566). Shoot number was increased by means of steady increase of BAP/TDZ concentration from 0.5 to 1.5 mg l^{-1} concentration in the regeneration medium, after 1.5 mg l^{-1} the shoot number was decreased (Table 2). The mean number of multiple shoots regenerated from mature embryo in different genotypes is given in the Figure 2. 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 that lead to the formation of multiple shoots.

Previous reports by Baskaran et al., (2006), Pola et al., (2007) also observed that, auxin and cytokinin combination will improve the regeneration. Gupta et al., (2006) suggest that, to overcome the genotypic limitations of plant regeneration in *Sorghum,* the callus induction medium must be supplemented with strong cytokinin like Kinetin with 2,4-D. Jha et al., (2009) also reported high frequency of shoot regeneration in *Pennisetum glaucum* using BA + 2,4-D in the regeneration medium.

Table 2 Effect of different concentrations and combinations of PGR on multiple shoot induction from immature embryo of *Sorghum bicolor*

Tablica 2. Utjecaj različitih koncentracija i kombinacija hormona rasta na indukciju višestrukih izboja iz nezrelog embrija sirka

Concentration of PGR Koncentracija hormona rasta (mg l^{-1})			Shoot number* Broj izboja	Root number# *Broj korjenčića*
BAP	TDZ	IAA		
0.5	-	-	12.2± 3.7	26.4±6.8
1.0	-	-	18.4±2.1	38±4.6
1.5	-	-	20.28±4.4	42±2.8
2.0	-	-	16.88±2.3	36±6.2
-	0.5	-	14.6±3.7	30±3.3
-	1.0	-	20.2±1.3	42±3.6
-	1.5	-	22.64±2.9	48±4.2
-	2.0	-	18.82±1.8	38±3.2
-	-	0.5	-	6±1.4
-	-	1.0	-	8±1.1
-	-	1.5	-	10±2.4
-	-	2.0	-	8±2.2
0.5	0.5	0.5	18.66±2.2	37.9±8.6
1.0	0.5	0.5	22.52±.1.6	46.2±6.4
1.5	0.5	0.5	29.18±2.4	63.2±2.8
0.5	1.0	1.0	34.12±4.2	78.4±6.3
1.0	1.0	1.0	36.22±6.2	80.66±4.2
1.5	1.0	1.0	38.24±2.8	84.6±2.8
0.5	1.5	10	39.62±3.6	84.82±6.4
1.0	1.5	1.0	40.42±2.8	86.6±8.4
1.5	1.5	1.0	43.22±4.6	94.4±6.2
2.0	1.5	1.0	27.55±4.2	66.82±4.6
2.0	1.0	1.0	14.77±3.4	68.62±2.8
2.0	0.5	1.0	20.66±1.8	44.2±2.6

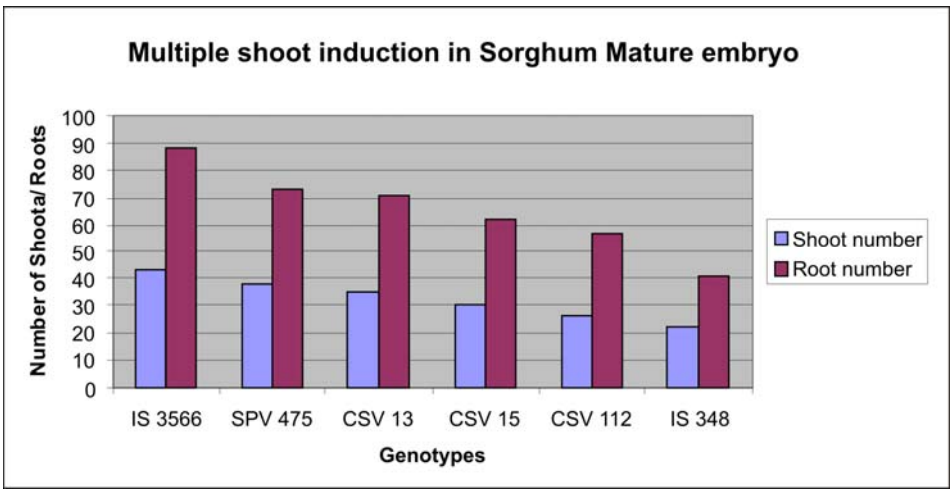
*Each value is an average number of 3 replications using 25 explants per replication. Mean number of shoots regenerated ± standard error (±SE)

**Svaka vrijednost prosjek je triju ponavljanja s po 25 eksplantanta. Prosječan broj regeneriranih izboja ± standardna pogreška (±SE)*

#Rooting medium - $1^{mg\ l^{-1}}$ NAA

#Medij ukorjenjavanja - $1^{mg\ l^{-1}}$ NAA

Figure 2 Multiple shoot induction in *Sorghum bicolor* from mature embryo* #
*Grafikon 2. Indukcija višestrukih izboja sirka iz zrelog embrija *#*



*Each value is an average number of 3 replications using 25 explants per replication
**Svaka vrijednost prosjek je triju ponavljanja s po 25 eksplantanta*

#Rooting medium - $1^{mg\,l^{-1}}$ NAA
#Medij ukorjenjavanja - $1^{mg\,l^{-1}}$ NAA

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 mg l^{-1} of BAP, TDZ with 1.0 mg l^{-1} IAA bestow enhanced multiple shoot production.

CONCLUSION

Efficient plant regeneration is a prerequisite for a complete genetic transformation protocol in cereals. In this report we have accomplish efficient plant regeneration using mature embryos as a source material in *Sorghum*. Mature embryos have always been ideal for *in vitro* studies for the reason that they can be handled easily

over other explants and available throughout the year. In mature embryo explant collection, we need not to sacrifice the plant but whereas with immature inflorescences we have to cut the plant to collect the explant. Although calli derived from immature inflorescence and immature embryos are best explant sources for *in vitro* culture, they are available only for a limited period in a year because of photoperiodic sensitivity of the *Sorghum* genotypes. Quick loss of regeneration potential in calli and problems associated with isolation and sterilization of immature embryos are the serious limitations for use of these explants in transformation.

In conclusion, an efficient and reproducible procedure for *Sorghum* mature embryos explant regeneration was developed in the present study despite the fact that *Sorghum* is considered to be the one of the recalcitrant species among the cereals for *in vitro* response. The successful use of mature embryos possibly will compensate for the shortage of immature inflorescence and immature embryos used in *Sorghum* transformation and, to a certain level, smooth the progress of future research of *Sorghum* genetic engineering experiments.

ZRELI EMBRIJ KAO POLAZNI MATERIJAL ZA UČINKOVIT POTICAJ REGENERACIJE KOD SIRKA (Sorghum bicolor L. Moench.)

SAŽETAK

Učinkovita regeneracija kod biljaka preduvjet je za potpun transformacijski protokol kod žitarica. Ovim istraživanjem izvedena je uspješna regeneracija biljaka korištenjem zrelih embrija sirka *Sorghum bicolor* (L.) Moench. kao polaznog materijala. Cvjetovi i nezreli embriji najbolji su za eksplantaciju *in vitro* kulture sirka, ali poteškoće se javljaju jer su oni dostupni samo u određenom dijelu godine. Zreli embriji su oduvijek bili idealni za *in vitro* istraživanja iz razloga što se njima puno lakše rukuje u odnosu na druge eksplatante, a dostupni su tijekom cijele godine. Zreli eksplatanti sirka genotipova IS 3566, SPV 475, CSV13, CSV15, CSV112 i IS 348 kultivirani su na MS mediju za stvaranje učinkovitog kalusa i naknadnu regeneraciju. U istraživanjima su testirane razne kombinacije i koncentracije regulatora rasta te faktori koji utječu na tkivo zrelog embrija. Uočene su značajne genotipske razlike kod indukcije embrionalnog kalusa i regeneracije biljaka. Genotip IS 3566 pokazao je bolje rezultate u kulturi tkiva od ostalih genotipa. Učinkovita indukcije embrionalnog kalusa postignuta je s 2mg l^{-1} 2, 4,5- Triklorfenoksioctenom kiselinom (2,4,5-T) i višestrukim dozama indukcije koje su postignute korištenjem 6-benzyl adenina (BAP), Thidiazurona (TDZ) i Indol-3-octenom kiselinom (IAA) u mediju kulture.

Ključne riječi: sirak, embriji, 2, 4,5-T, embriogenetski kalus, BAP, TDZ, regeneracija

REFERENCES - LITERATURA

1. Asad, S., Muhammad, A., Shahid, M.,Yusuf, Z. 2009. Effect of various amino acids on shoot regeneration of sugarcane (*Sacchrum officinarum* L.). African Journal of Biotechnology. 8(7):1214-1218._www.academicjournals.org/AJB/PDF/pdf2009/6Apr/Asad%20et%20al.pdf
2. Aparna, G., 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. http://www.springerlink.com/index/K676H46215275LN1.pdf
3. Bi, R., Kou, M.M., Chen, L.G., Mao, S.R., Wang, H.G., 2007. Plant regeneration through callus initiation from mature embryo of *Triticum*. Plant Breed. 126, 9-12. DOI_10.1111/j.1439-0523.2007.01327.x
4. Baskaran, P., Rajeswari, B. R., 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. http://journals.tubitak.gov.tr/botany/issues/bot-06-30-1/bot-30-1-1-0502-3.pdf.
5. Bhaskaran, S., smith, R.H., Schertz, K.1983. Sodium chloride tolerant callus of Sorghum bicolor (L.) Moench. Z. Phanzenphysiol.Bd.112:459-463.
6. Chang, Y., Zitzewitz, J., Hayes, P.M., Chen, T.H.H. 2003. High frequency plant regeneration form immature embryos of elite barley cultivars (*Hordeum vulgare* L. cv. Morex). Plant Cell Rep. 21: 733–738.
7. Eudes, F., Acharya, S., Laroche, A., Selinger, L.B., Cheng, K.J. 2003. A novel method to induce direct somatic embryogenesis, secondary embryogenesis and regeneration of fertile green cereal plants. Plant Cell Tiss. Org. Cult. *73*: 147–157.
8. Gao, Z., Jayaraj, J., Muthukrishnan, S., Claflin, L., Liang, G.H., 2005. Efficient genetic transformation of *Sorghum* using a visual screening marker. Genome. 48: 321–333.
9. Gupta, S., Khanna, V.K., Rameshwar, S., 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 Organ Culture. 86: 379–388.
10. Gupta, S. D., 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. http://www.springerlink.com/index/M103K617713G735X.pdf
11. Hagio, T., 1994. Varietals Difference of plant Regeneration from callus of *Sorghum* mature seed. Breed. Sci. 44:121-126.
12. Jha, P., Yadav, C. B., Anjaiah, V., Bhat,V. 2009. In vitro plant regeneration through somatic embryogenesis and direct shoot organogenesis in Pennisetum glaucum (L.) R. Br. In Vitro Cell.Dev.Biol.-Plant. 45:145–154. 45:145–154.DOI 10.1007/s11627-009-9198-6.
13. Jogeswar, G., Ranadheer, D., Anjaiah, V., KaviKishor, P.B. 2007. High frequency somatic embryogenesis and regeneration in different genotypes of Sorghum bicolor (L.) Moench from immature inflorescence explants. In Vitro Cell Dev. Biol. 43: 159–166.DOI 10.1007/s11627-007-9033-x
14. Kishore, S.N., Visarada, K.B.R.S., Lakshmi, A.Y., Pashupatinath, E., Rao, S.V., Seetharama, N. 2006. *In vitro* culture methods in *Sorghum* with shoot tip as the explant Material. Plant Cell Rep. 25: 174–182. DOI 10.1007/s00299-005-0044-y
15. Manjula, S.M., Kuruvinashetti, M.S., Harti, C.C., 2000. Regeneration establishment and evaluation of soma clones in *Sorghum bicolor* (L.) Moench. Euphytica. 115, 173-180.
16. Mikhail, F., Dmitry, M., Darya, V., Sergey, D. 2006. The effect of auxins, time exposure to auxin and genotypes on somatic embryogenesis from mature embryos of wheat. 84(2) 213-222. Plant Cell, Tissue and Organ Culture. DOI 10.1007/s11240-005-9026-6.
17. Miller, D.R., Waskom, R.M., Duncan, R.R., Chapman, P.L., Brick, M.A., Hanning, G.E., Timm D.A., Nabars,M.W. 1992. Acid soil stress tolerance in tissue culture derived *Sorghum* lines. *Crop Sci.*32:324-327.
18. Monnier, M. 1990. Zygotic embryo culture. In: Bhojwani SS (ed) Plant tissue culture:Applications and limitations. 366-393. Elsevier, Amsterdam.

19. Murashige, T. Skoog, F. 1962.A revised medium for rapid growth and bioassays with Tobacco tissue cultures. Physiol. Plant. 15: 473–497._10.1111/j.1399-3054.1962.tb08052.x
20. Nguyen, T.Van., Tran, T. T., Artine, C., Geert, A.,2007. Agrobacterium-mediated transformation of sorghum (Sorghum bicolor (L.) Moench) using an improved in vitro regeneration system. Plant Cell Tiss Organ Cult. 91:155–164.DOI 10.1007/s11240-007-9228-1.
21. O'Kennedy, M. M., Burger, J. T., Botha, F. C. 2004.Pearl millet transformation system using the positive selectable marker gene phosphomannose isomerase. Plant Cell Rep. 22: 684–690. doi:10.1007/s00299-003-0746-y.
22. Pola, S. 2005.Tissue culture and genetic transformation studies of Sorghum bicolor *Ph.D. Thesis, college of science and technology*, Andhra University, Visakhapatnam, INDIA. pp 18-28.
23. Pola, S., Saradamani, N., Ramana, T. 2007. Enhanced shoot regeneration in tissue culture studies of *Sorghum bicolor*. Journal of Agricultural Technology 3(2): 275-286. http://www.ijat-rmutto.com/pdf/Nov_V3_no2_07/11-IJAT2007_20-P%20275-286.pdf
24. Ratnavati, C.V., Dayakar, B., Seetarama, N. 2003. Sweet *Sorghum* stalks: A suitable raw material for fuel alcohol production. *NATP Research Bulletin*, National Research Centre for *Sorghum*, Rajendranagar, Hyderabad, India, pp 8
25. Sasaki Takuji., Baltazar, A. Antonio. Sorghum in sequence. 2009. Nature. 457:547-548. doi :10.1038/457547a.
26. Shan, X., Li, D., QU, R. 2000. Thidiazuron promotes in vitro regeneration of wheat and barley. *In vitro* cellular Developmental Biology of Plant 36:207-210. http://www.springerlink.com/ index/D8704G8361LN2105.pdf
27. Smith, R.H., Bhaskaran, S., Schertz, K. 1983. Sorghum plant regeneration from aluminum selection media. Plant cell reports. 2:129-132.
28. Sudhakar, Pola., Sarada Mani, N., Ramana, T. 2008. Plant tissue culture studies in *Sorghum bicolor*: immature embryo explants as the source material. International Journal of Plant Production. 2 (1): 1-14. http://gau.ac.ir/journals/ijpp/showpdf.php?id=279
29. Sudhakara, R Pola., Saradamani, N.2006. Somatic embryogenesis and plantlet regeneration in *Sorghum bicolor* (L.) Moench, from leaf segments. Journal of cell and molecular biology. *Journal of Cell and Molecular Biology* 5: 99-107.http://jcmb.halic.edu.tr/pdf/5-2/Somatic.pdf
30. Waskom, R.M., Miller, D.R., Hanning, G.E., Duncan, R.R., Voigt, R.L., Nabors, M.W. 1990. Field evaluation of tissue culture derived sorghum for increased tolerance to acid soils and drought stress. Can.J. plant sci. 70: 997-1004.

Author's address – Adreasa autora
Dr. Sudhakar Pola
Faculty of Plant tissue culture
Department of Biotechnology
Andhra University
Visakhapatnam, INDIA 530 003.
E-mail: sudhakarpola@gmail.com

Primljeno – Received:
17. 08. 2009.