ISSN:2249-1236

INTERNATIONAL JOURNAL OF RESEARCH AND REVIEWS IN PHARMACY AND APPLIED SCIENCES

Research Article

Received: 06-08-2011 *Accepted: 12-09-2011* *Published: 29-09-2011*

OPTIMIZATION OF REGENERATION PROTOCOL FOR MAIZE (ZEA MAYS L) FROM CALLUS

M.Guruprasad[1], SK.Jaffar[2], P.Rajakumar[3], E. Muralinath[3], D.Sandeepraja[3].

[1]Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Tirupati-517 502, A.P, India, [2]Dept of Biochemistry, Acharya Nagarjuna University, Guntur-522 510, A.P, India, [3]Dept of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram- 521 102, A.P, India.

Corresponding Author

SK. Jaffar
Guntur, AP, India
shaikjaffar2008@yahoo.com

ABSTRACT

An efficient regeneration procedure was developed for maize from mature embryos derived from callus of maize inbred line. We found that 3mg/l 2-4 D in MS medium was optimum for the callus induction .the induction frequency of primary calli was over 85%. Efficient shoot regeneration was obtained on regeneration medium containing 0.5mg/l BAP in combination with 0.5 mg/l kinetin. Regenerated shoots were rooted on half strength MS medium containing 1mg/l IBA. This plant regeneration protocol provides a foundation for genetic transformation of maize.

Key words: Maize, MS medium, calli, embryogenesis, Agrobaterium.

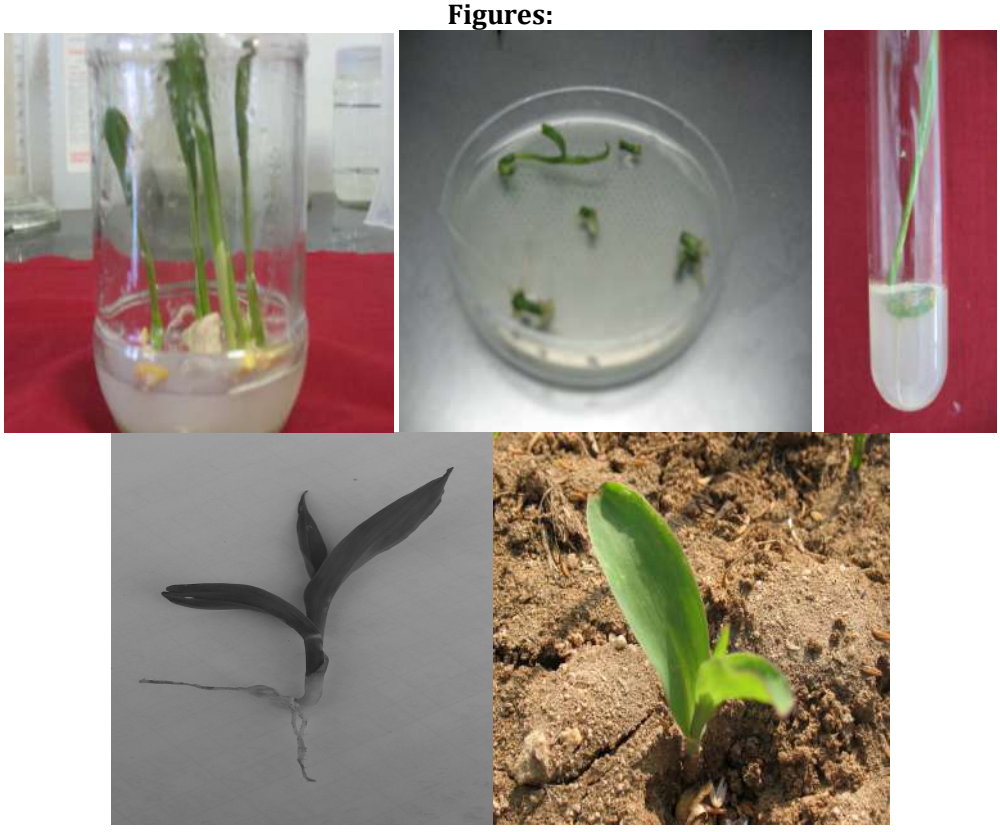
INTRODUCTION

Maize (*Zea mays*) is one of the widely growing important cereal crops (Smith RA.*et al*., 1990) in the world. With the increasing population and limitation of land, the great demand for maize both quality and quantity requires more rapid genetic improvement. Consequently, several biotechnology approaches have received more emphasis and genetically transformed maize plants have been obtained by various approaches, such as particle, bombardment (Klein *et al.,* 1989) and Agro-bacterium mediated (Schnapps and Hahn 1992; Valdei-or-tiz *et al.,*2007) immature calli is efficient than the other calli. Immature embryo availability is difficult in all seasons. But the regeneration from the mature embryos (Huang X Q,Wei *et al*.2004) can be seen in some genotypes only .But, there is a need to develop efficient regeneration system from excised mature embryos of maize seed to assist in more efficient genetic improvement of this crop.

MATERIALS AND METHODS

Mature embryo was derived from the inbreed lines. Seeds were surface sterilized with 70% ethanol for 5 minutes and then followed by 0.1% mercuric chloride for 15 minutes .Later on washed with distilled water thrice .After that there were softened by soaking in the sterilized water overnight . Mature embryos were dissected out from plumages on scutellar nodes.

Figures:



CALLUS INDUCTION

Mature embryos were cultured on MS medium (Murashige. T, and Skoog, F. *et al.*,1962) supplemented with 30g/l sucrose and different concentrations of 2-4 D like (1,2,3,4,5mg/l) medium was solidified with 0.8% agar .The P^H was adjusted to 5.8 before autoclaving for 20 minutes at 15 lb .After four weeks of culture in darkness at 27^0C compact and light yellow colour callus were collected and sub cultured (George,E.F. *et al.,* 1996) on same medium for maintain and proliferation .The cultures grown in dark at 27^0C and transferred every 2 weeks for fresh medium.

PLANT REGENERATION:

The embryogenesis calli(Erdelsk K E.*et al*.1987) were transferred on to medium containing various concentrations of BAP (0.1, 0.2, 0.3, 0.4, 0.5, 0.6,0.7, 0.8 ,0.9 and 1.0) and in combination with kinetin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6,0.7, 0.8 ,0.9 and 1.0).The cultures were grown in 16 hours photoperiod with light intensity of 60µ $mol.m^2\ s^1$

Root induction:

Regenerated shoots of 3 cm high were transferred to the rooting medium (half strength MS basal medium with 1mg/l IBA).After 3-4 weeks ,plantlets with well developed roots were transferred to pots and transplanted in a growth room.

RESULT AND DISCUSSION

Callus induction and proliferation:

Within 4 days callus initiation was taken from the cultured embryo surface. The range of 32.3% to 90.5 % callus induction frequency of primary callus (Widholm, J. M.*et al*.1985) was observed due to the effect of 2-4 D .The optimal level of 2-4 D for callus initiation was 3mg/l.Among the different concentrations of the proline (.Lu C, Vasil .*et al*.1983), 12µM was proved to be the optimal concentrations for embryogenic callus proliferation.(jho cheng-hao *et al*.2008).

Shoot regeneration:

Shoot regeneration depends on selection of concentration and kind of growth regulator (Thorpe T A.*et al*.1996) in plants. so, we tested different concentrations of cytokines in MS medium .The plantlet regeneration (Plillips.*et al*.1975) was seen in hormone free medium within 2 weeks and we found that addition of BAP and Kinetin enhanced the plantlet regeneration (J.W.Van Dun.*et al*.2005) .Among the combination tested 0.5mg/l BAP and kinetin were the most effective(Orlikowska TK,Der WE *et al*.1993).

Root induction:

Among the different concentrations of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6,0.7, 0.8 ,0.9 and 1.0) IBA we had better result in 1mg/l.Even NAA and IAA combination was taken.

Effect of benzyl amine purine in Callus

s.no	Growth hormone Bap	kin	No of explants	Percent of explants responded
1	0.1	0.1	8	Callus
2	0.2	0.2	8	Callus
3	0.3	0.3	8	Callus
4	0.4	0.4	8	40
5	0.5	0.5	8	80
6	0.6	0.6	8	40
7	0.7	0.7	8	Callus
8	0.8	0.8	8	Callus
9	0.9	0.9	8	Callus
10	1.0	1.0	8	Callus

Effect of indole butyric acid on rooting

S.No	Growth regulator In dole butyric acid	No of explants	Rooting percent
1	0.1	20	10
2	0.2	20	22
3	0.3	20	35
4	0.4	20	55
5	0.5	20	60
6	0.6	20	65
7	0.7	20	72
8	0.8	20	80
9	0.9	20	85
10	1.0	20	92

CONCLUSION

In future this plant regeneration protocol provides novel foundation for genetic transformation of maize. It can be helpful to efficient regeneration from callus of maize inbred lines.

ACKNOWLEDGEMENT

Author thankful to regional Agriculture research station and Dept of Biochemistry to provide all necessary facilities to carry out experiments

REFERENCES

1. Bhaskaran S, Smith RA.1990.Regeneration in cereal tissue culture: a review .crop Science, 30, 1328-1336.
2. Conger B V, Novak F J, Afza R, Erdelsk K E.1987.Somatic embryogenesis from cultured Leaf segments of Zea mays.Plant cell Reports, 6,345-347.

3. Duncan D R,Williams M E, Zahra B E,Widholm J M.1985.The production of callus Capable of plant regeneration from immature embryos of numerous Zea mays genotypes.Planta, 165, 322-332.
4. Gaspar T,Kevers C,Greppin H,Reid D M,Thorpe T A.1996.Plant hormones and plant Growth regulators in plant tissue culture. In vitro cellular & Developmental Biology-plant ,, 32,272-289.
5. Green C E, Plillips R L.1975.Plant regeneration from tissue culture of maize. Crop Science, 15,417-421.
6. Huang X Q, Wei Z M.2004.High –frequency plant regeneration through callus initiation From mature embryos of maize (*Zea mays* L.)Plant cell Reports, 22,793-800.
7. Lu C, Vasil I K.1983.Improved efficiency of somatic embryogenesis and plant Regeneration in tissue culture of maize (*Zea mays* L.).Theoretical and Applied Genetics, 66,285-289.
8. Ting C, u M, Zheng W Z.1981.Improved anther culture of maize. Plant science Letters, 23,139-145.
9. Greenplate, J.T., J.W.Mullins,S.RPenn,A.Dahm,B.J.Reich,J.A.Osborn,P.r.Rahn,L.Rusch Ke and Z.W.Shapple.2003.Partial characterization of cotton plants expressing two toxins Proteins from Bacillus thuringiensis: relative toxin contribution, toxin interaction, and Resistance management.J.Appl.Entomol.127:340-347.
10. Jackson, R.E., J.R.Bradle, and J.W.Van Dun.2005. Comparative efficacy of Bt technologies against bollworm in North Carolina,pp.1373-1378.In Proceedings of the Belt wide Cotton Conference,4-7Januar 2005,New Orleans, National cotton council of America,Memphis,TN. Halamkova,E., Vagera,J. ,Ohnoutkova, L.: Regeneration capacity of calli derived from Immature embryos in spring barley cultivars-Biol.Plant.48:313-316, 2004.
11. Murashige T and Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures.Phsiol plant, 15(1962)473-497.
12. Orlikowska, T.K, Der, W.E.1993.In vitro regeneration and multiplication of safflower (Carthamus tinctorius L.) Plant science 93:151-157.
13. George, E.F.1996.Plant propagation b tissue .part 2.Exegetics Ltd., Edington,UK.
14. Finer, J.J.Apical proliferation of embryogenic tissues of so bean {Glycine Max (L.)Merrill].Plant cell Rep.7:238-241:1988.