ANNQUEST December 2014

ISSN: 2321-3043

www.stannsannquest.com

High frequency somatic embryogenesis and plantlet regeneration from shoot tip explants of Soy bean (*Glycine max* .L.) Merr.)

*** 1 2**

R.Chakravarthy and T. Ugandhar

Abstract:

Establishment of efficient protocol for the induction of somatic embryogenesis and plantlet regeneration from shoot tip explants was achieved in *Glycine max* (L).Merr. cv.PK472. Highest frequency (95%) of embryogenic callus formation was obtained from shoot tip explants of *Glycine max* (L).Merr.cv.PK472 on Murashige and Skoog (MS) medium fortified with different concentration of 2,4-Dichlorophenoxy acetic acid (2,4-D) and Benzyl amino purine (BAP) .Fast growing, yellowish nodular callus lines containing somatic embryos were established on initiation medium supplemented with 5.0mg/L 2.4-D and 1.0mg/L BAP respectively. Embryo development and maturation was achieved on MS medium supplemented with 5.0mg/L 2,4-D and 4.0mg/L BAP .The well formed embryos germinated into complete plantlet on MS medium containing 4.0mg/L BAP and 1.0mg/L NAA(Napthalene acetic acid).The regenerated plants ware first transferred to plastic cups and later into pots for

1.Dept. of Biotechnology, K L University, Green Fields, Vaddeswaram – 522502, Guntur,A. P *corresponding author drrchakravarthy@gmail.com

2. Dept. of Botany, SRR Degree College, Karimnagar

maturation. Regenerated plants did not exhibit observable morphological alternation.

Key words: Shoot tips, callus, somatic embryogenesis, plant regeneration.

Introduction:

Legumes are a group of economically important plants valued for food, fodder, wood, ornamentals, and raw materials for industry and also for their role in biological nitrogen fixation(Duke J.A 1981) .Grain legumes are a major source of proteins for more than two billion people worldwide. Plant transformation technology has gradually become a useful tool for cultivar improvement as well as for studying gene function .A reliable regeneration system to be precise, is universally required in most currently available transformation protocol to recover pants .Plants are regenerated from cell or callus culture either through organogenesis or somatic embryogenesis (Hansen G etal 1999).

Soybean [*Glycine max* (L.) Merr] in one of the major grain legumes of tropical and subtropical regions and in grown for its high seed protein and oil contents. *In vitro* regeneration of plants via somatic embryogenesis has much potential for plant propagation and gene transfer(Sato S et al 1993). In soybean somatic embryos have been obtained from cultured immature cotyledons (Lippman 1984) leaf and stem (Ghazi TD1986), cotyledonary node Kerns HR etal1986) and another (Santos 1997) Some of the serious limitations of the somatic embryo genesis protocols are the low frequency, inconsistency, genotype specificity and occurrence of callus phase prior to embryogenesis. The author reports here a protocol for *in vitro* embryogenesis and plant regeneration of soybean from embryogenic callus line derived from the shoot tip explants.

In this communication, we reported a high frequency regeneration system in *Glycine max* (L) through direct somatic embryogenesis from shoot tip explants using BAP, without involving callus phase.

Seeds of *Glycine max* (L.) CV PK 472 obtained form ICRISAT, Hyderabad, were surface sterilized with 0.5% mercuric chloride (w/v) for 5 minutes and then rinsed distilled water 4-5 times. The seeds were germinated on sterile moist filter paper in pariplate/sterile moist cotton in flasks at 22-25^{0}C in dark. Shoot tip segments excised form 25-d-old seedlings and placed aseptically on a solidified MS medium containing 3% sucrose and 0.8% agar agar. A total of 48 shoot tip explants were cultured on the media. The pH of the media was adjusted to 5.6-5.8 with 0.1N sodium hydroxide and /or 0.1N hydrochloric acid prior to adding the agar. The media were supplemented with filter sterilized auxin 2.4-Dand cytokinin 6-benzylaminopurine (BAP) in combination at a concentration of 2.4-D (1.0-7.0mg/L) and BAP (1.0mg/L) cultures were maintained under white fluorescent light (40-50µEm^{-2}.sec^{-1}) at a cycles of 16th/8th at 25±2^{0}C. The end of 7 weeks, percentage of explants with somatic embryogenesis (explants with globular and later stages of embryos) was recorded.

Results and Discussion:

Globular embryos formed after 6 weeks of culture were separated form the callus and transferred to (Murashige T & Skoog F 1962)MS basal medium augmented with 5.0mg/L 2.4-D and different concentrations of BAP (1.0-6.0mg/L)for further development and maturation of somatic embryos, cultures were incubated at 26±2^{0}C under the same condition as for callous induction.

For germination, globular or cotyledonary embryos were transferred onto MS^8 medium containing BAP (1.0-6.0mg/L) and 1.0 mg/L NAA. The embryos were incubated for 15d at 25±2^{0}c under continuous illumination (35 mol m^{-2} s $^{-2}$)

with fluorescent lighting. They were transferred to test tubes (25X15) containing 15 ml of the MS medium with out plant growth regulators. Regenerated plantlets with well developed shoots and roots were removed from the culture medium washed gently under running tap water and transferred to plastic cups containing vermin culture, which was maintained under culture conditions initially and subsequently established in pots under field condition.

Callus was initially mainly from the cut ends of the shoot tip explants in contact the medium followed by 35-45 days of culture .A highest frequency (95%) of embryogenic calli induction was observed on MS medium augmented with 5.0mg/L 2,4-D in combination with 1.0mg/L BAP.(Table-1 Fig1-a). It was reported that in other studies of soybean 2,4-D has been the most commonly used auxin for induction of somatic embryogenesis (Randi JP et al 1985). In the present study in media supplemented with 2,4-D(3.0-7-0mg/L), clusters of somatic embryos developed from the embryogenic callus of the explant (Fig 1b).Addition of 4.0mg/L BAP in combination with 1.0mg/L BAP promoted growth, development and maturation of somatic embryos. The highest embryo maturation response recorded was 85.6% (Table 2 Fig 1c).Positive effect of the cytokinins on somatic embryogenesis in soybean was also reported (Lazzari PA 1987 & ian LNM 1994) It was also reported that as the auxin concentration increased, the probability of obtaining a normal shaped embryo decreased (McKently1991)

Table1

Effect of Different concentration of 2,4-D in combination with BAP on induction of Somatic embryogenesis from shoot tip explants of *Glycine max* cv PK-472.

Growth regulators mg/L	% of somatic embryogenesis	Mean no of embryos per explant
MS+2,4-D		
1.0	50	15.9 + 0.27
2.0	65	25.0 ± 0.35
3.0	80	35.2 ± 0.13
4.0	84	68.0 ± 0.35

5.0	95	50.3 ± 0.45
6.0	75	40.0 ± 0.29
7.0	70	32.5 ± 0.33

Table – 2

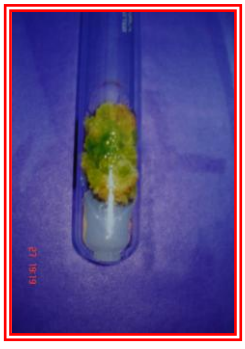
Effect of BAA in combination with 5.0 mg/L 2,4-D on embryo maturation in *Glycine max* cv PK-472

Growth regulators mg/L MS+BAP	% of Embryo maturation response
1.0	34.7±0.36
2.0	50.8±0.37
3.0	60.4±0.27
4.0	85.6±0.37
5.0	70.3±0.27
6.0	45.4±0.37

Table -3

Effect of different concentration of BAP in combination with 1.0mg/LNAA on germination of somatic embryos derived from shoot tip explants of *Glycine max* cv PK-472

Growth regulators mg/L MS+BAP	Number of embryo germination	% of Embryo germination response
1.0	20	18.4±0.22
2.0	28	35.0±0.25
3.0	48	52.5±0.35
4.0	65	82.0±0.92
5.0	40	60.0±0.32
6.0	30	40.0±0.32



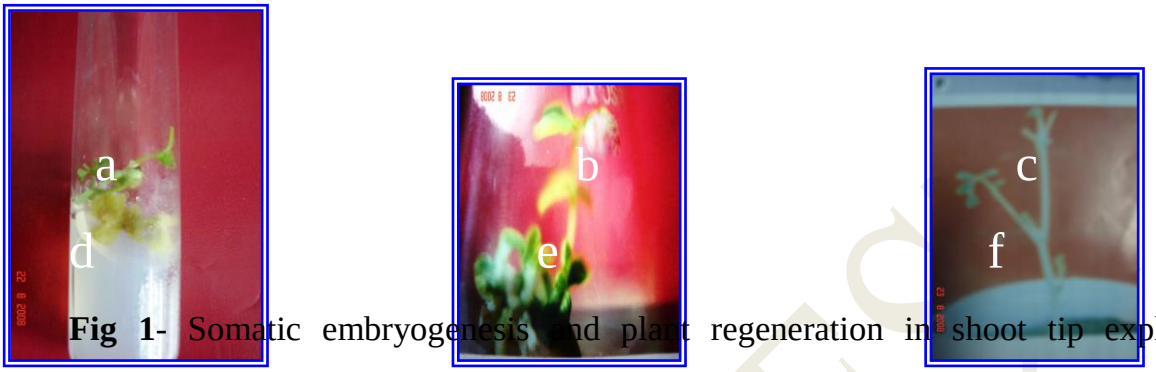


Fig 1- Somatic embryogenesis and plant regeneration in shoot tip explants cultures of *Glycine max* L.(Merr) a) embryogenic callus induction b) callus showing early stage embryos c) maturation of somatic embryos d) germination of somatic embryos, e) plant with normal shoot and root system developed from somatic embryos f) regenerated plantlet growing in plastic cup.

.Higher concentration of auxin not only decreased the number of embryos but also delayed embryogenesis (Reddy RI etal 1993).

Up on transfer to a media containing 4.0mg/L BAP and 1.0mg/L NAA, globular or cotyledonary stage embryo germinated. They turned green elongated and revealed the folded cotyledon in the germination medium .Later the cotyledons unfolded the shoot region is seen with the embryogenesis of the first leaves. The combination of 4.0 mg/L BAP and 1.0 mg/L NAA was found to be the best for highest frequency (85.6%) of embryo germination (Table-3 fig 1d), Germination of embryos was characterized by the simultaneous production of shoot and root systems on transfer to MS based medium (Fig 1e) Earlier workers could achieve only a very low plant conversion frequency (Buchheim JA 1989) the efficiency of somatic embryos germination in *Glycine gracilis* (Komatsudha et

al 1992)was 60-64% But in the present study, a highest germination rate 82% was achieved.

Plant lets with well developed shoot and root systems were washed carefully with tap water and transferred to plastic cups containing a mixture of vermiculite and soil for hardening (Fig1f). The acclimatized plantlets were successfully transplanted to pots under field conditions. Regenerated plants showed no observable morphological alteration.

Acknowledgement We are grateful to Dr.S.Vijaya Saradhi, HOD Biotechnology Dept. and Prof G.L Datta Vice chancellor for their encouragement.

References

1 Duke J.A., *Handbook of legume of world Economics importance.* Plenum press, Newyork , 1981.

2 Hansen G and Wright M.S. Recent advances in the transformation of plants, *Trends plant Sci.* 4 (1999) 226-231.

3 Sato S, Newell C, Kolacz K and Tredo L, Stable transformation via particle bomardment in two different soybean regeneration systems *Plant cell Rep.* 12, (1993) 408-413.

4 Lippman B. and Lippmann G. Induction of somatic embryogenesis in cotyledonary tissue of soybean, *Glycine max.* (L) Mess, *Plant cell Rep,* 3 (1984). 215-218.

5 Ghazi T.D. Cheema H.V. and Nabors MW, Somatic embryo genesis and plant regeneration from embryonic callus of soyabean (*Glycine max*(L). Marr.), Plant physiol, 77(1986) 863-868.

6 Kerns H.R., Barwale VB and Meyer MM, Correlation of cotyledonary node shoot proliferation and somatic embryoid development in suspension culture of soybean (*Glycine max* (L) Merr..) *Plant cell Reports* (1986). 140- 143.

7 Santos EK., Mundstock E. and Bondanse- Zanetti M H., Cytological analysis of early microscope divisions and embryo formation in cultured soybean anthers, *Plant cell Tissue organ Cult.* 219(1997) 107- 115.

8 Murashige T. and Skoog F, A revised medium for rapid growth and biassays with tobacco tissue cultures, *Physiol Plant* 15 (1962) 477-497.

9 Ranch JP, Ogelshy L and Zielinski AC, Plant regeneration of somatic embryos from all suspension cultures of soybean *In vitro cell Dev Biol.* 21 (1985) 653- 658.

10 Lazzeri P.A. Hilderbr and DF and Collins GB, soybean somatic embryogenesis: Effects of hormones and culture manipulations *Plant cell Tissue organ cult.*10 (1987) 197-200.

11 Tian LNM, Brown PL, Voldeng H and Webb J. *In vitro* response and pedigree analysis for somatic embryo genesis of long day photoperiod adopted soybean, *Plant cell Tissue Organ cult,* 36, (1994) 269-273.

12 Mc Kently AH, Direct somatic embryogenesis from axes mature preenut embryos *In vitro cell Dev Biol,* 27 (1991) 197-200.

13 Reddy RI and Reddy GM, Factors affecting direct somatic embryo genesis and plant regeneration in ground nut (*Arachis hypogea* L.) *India J. Exp Biol,* 33 (1993) 57- 60.

14 Buchheim JA., collburn SM and Ranch JP. Maturation of soybean somatic embryos and the transition of plant growth, *Plant physiol* 15, (1989) 768-775.

15 Komatsudha T., Wenbin L. and Scibi O., Maturation and germination of somatic embryos as affected by sucrose and plant growth regulators in soybean (Glycine gracilis SK vortz and *Glycine max (*L Merr.) *Plant cell Tissue Organ Cult* 28,(1992) 103-113.