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Research Article

SELECTION OF SOMACLONES RESISTANT TO ALTERNARIA BLIGHT IN SESAME (*SESAMUM INDICUM* L.) THROUGH *IN VITRO* CELL LINE METHOD

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ABSTRACT
To achieve somaclones resistant to *Alternaria* blight of sesame, *in vitro* cell line selection was carried out by incorporating partially purified pathotoxin, on an incremental basis of 100 ppm, starting from 100 to 1000 ppm concentrations derived from culture filtrate of pure culture of *Alternaria sesami* pathogen to MS media supplemented with NAA @ 0.5 mg/l, BAP @ 1.5 mg/l and Kinetin @ 1.5 mg/l using the callus of six susceptible genotypes (E8, Gulbarga locals white and brown, Tumkur locals white and brown and W-II) and one resistant genotype (RT-273; identified resistant through field screening). Calli of susceptible genotypes survived only up to 300-ppm concentration whilst the callus of resistant genotype survived up to 700-ppm. The callus of RT-273 survived and proliferated in all the bottles when co-cultivated with the calli of all susceptible genotypes which died at 400 ppm concentration. Calli of all genotypes were dead when exposed to UV rays for six hrs continuously. A total of 33 calli were recovered when callus of susceptible genotypes were exposed to UV rays for one hr followed by plating on MS media supplemented with 1000 ppm, of which only 15 somaclonal calli responded for sub-culture. Regeneration is underway. The difference in response has been discussed in the light of intrinsic resistant nature of RT-273 genotype.

***Key Words**: Alternaria Sesami Pathotoxin, Culture Filtrate, Sesamum Indicum, Somaclones*

INTRODUCTION

In vitro cell line selection, a non-conventional approach, has been proved to be a rapid and reliable technique to develop resistant lines against biotic stresses involving partially purified toxin that serves as selection pressure using callus of susceptible genotypes with or without induced mutagenesis. It has been well utilized in crops of economic importance as well as in other oil seed crops (Larkin *et al*., 1984; Venkatachalam and Jayabalan, 1996; Janagoudar, 2000; Ashok, 2001 and Kariyallappa, 2003). The usage of *in vitro* cell line selection in sesame is in infancy. A few workers have used to create somaclones that are resistant to herbicide, charcoal root rot and wilt disease complex and *Alternaria* blight using calli (Chae *et al*., 1987; Kim *et al.,* 1987; Moneem *et al*., 1997; Kariyallappa, 2003). For instance, Chae *et al*. (1987) reported *in vitro* selection for herbicide tolerant calli and plant regeneration in sesame, whilst Moneem *et al.* (1997) recorded *in vitro* selection of callus, using sesame variety Giza 25, screened against charcoal root rot (causal agents: *Sclerotium bataticola*, *Macrophomina phaseolina*) and wilt disease (*Fusarium oxysporium* sp. *sesami*) and succeeded in generating 112 Somaclones of which 52 were resistant. Kariyallappa (2003) made an attempt to screen callus of highly susceptible genotypes for *Alternaria* blight incorporating different proportion of culture filtrate of *Alternaria sesami* fungus by volume basis and succeeded in generating a few somaclones that could survive at higher concentration, however, failed to regenerate.

Sesame (*Sesamum indicum* L.), nicked as queen of oil seed crops, one of the most ancient oil seed crops known to man, native to Africa (Anonymous, 1990), occupies fifth position in the world with an area of

6.7 million ha with 2.5 million tones of production (Anonymous, 2004). India has the largest area (1.81 million hectares) and is the highest producer (8.03 lakh tones) in the world with a productivity of 453 Kg/ha. India also earns a sizable foreign exchange through export of white seeded sesame globally. Sesame oil is highly valued owe to long shelf-life due to presence of antioxidants *viz*. sesamin and sesamol, also contains about 47 per cent oleic and 39 per cent linoleic acids.

Regardless of these privileges, the crop improvement of sesame is not rapid. *Alternaria* blight, caused by *Alternaria sesami* and *A. alternata* has been identified as a predominant biotic pressure, limits yield and quality of sesame considerably. Most cultivated/ popular genotypes are highly susceptible to *Alternaria* blight and the severity has been recorded to be >80% in North-Eastern Karnataka.. Breeding for *Alternaria* resistance is recognized as a thrust area. But the resistance in cultivated types is highly scarce. To induce resistance in popular local varieties, an innovative approach of, *in vitro* cell line selection method was thought supplemented with in vitro mutagenesis using UV rays, hence the present investigation.

MATERIALS AND METHODS

Callus induction was achieved from six *Alternaria* blight susceptible genotypes *viz*. Gulbarga locals – white and brown, Tumkur locals – white and brown, E-8, Western II and an *Alternaria* resistant genotype RT-273 (identified through field testing by Naik *et al*., 2003 (Table 1)), on MS media supplemented with 0.5 mg/L NAA, 1.5 mg/L Kinetin and 1.5 mg/L BAP, the media composition standardized by earlier workers (Shashidhara, 2005; Lokesha *et al*., 2005). The toxin of *Alternaria sesami* was obtained by following the method described by Bhaskaran and Kanda Swamy (1978) and adopted by Savitha (2004) for *Alternaria sesami*. Partially purified toxin was crystallized into powdered form (Fig. 2) and a standard solution of 2000 ppm concentration was obtained by dissolving 100 mg powdered toxin in 50 ml sterilized distilled water (Naik *et al.,* 2005). The investigation was conducted in four different experiments.

In the first experiment, uniform pieces of calli of all the genotypes were placed on MS media supplemented with varying concentrations of toxin (100 to 1000 ppm with 100 ppm increment) along with resistant genotype (RT 273) as control to assess the intrinsic tolerance level of different varieties. Observation was collected on the survival of callus across concentrations after 15th day. Second experiment was co-cultivation of calli, on MS media with 400-ppm concentration of susceptible genotypes along with the callus of RT-273. Callus survival and proliferation was recorded on 15th day to confirm the callus survival of susceptible varieties as observed in first experiment. In the third experiment, the callus of all genotypes were exposed to UV rays at different time intervals to record maximum duration of exposure required for complete death of callus. In the fourth and final step, the callus of susceptible genotypes were exposed to UV rays for one hour and were plated on MS media supplemented with 1000 ppm toxin concentration to recover somaclones. The somaclones survived were sub-cultured on MS media with 0.5 mg/L NAA, 1.5 mg/L Kinetin and 1.5 mg/L BAP with 1000-ppm pathotoxin as per Lokesha *et al*., (2008). Whole plant regeneration is being attempted.

RESULTS AND DISCUSSIONS

The callus of all susceptible genotypes survived up to 300 ppm load of toxin (Fig. 3) whilst RT-273 callus alone survived up to 700 ppm (Fig. 4) confirming the field observations with respect to resistant nature of RT-273 sesame genotype against *Alternaria* fungal infection (Figure 4). The callus of RT-273 survived and proliferated whilst that of susceptible types died when co-cultivated at 400 ppm concentration of culture filtrate (Figure 5; Fig 5a & 5b). Complete death of callus, irrespective of genotypes, was observed for 6 hours exposure to UV rays (Fig 1). However, a good number of somaclones were induced in 1000 ppm concentration after exposing calli of all susceptible genotypes to UV rays for one hour duration followed by plating on MS media (Table 2).

In vitro cell line selection in particular and plant tissue, cell and organ culture in general have opened up numerous new possibilities in several crops *vis* a *vis* sesame (Ram *et al*. 1990). *In vitro* screening for disease resistance at cellular level using toxins has been proved very useful (Larkin *et al*., 1984; Venkatachalam *et al*., 1998; Janagoudar, 2000; Ashok, 2001). Plant tissue culture can play a significant role for the enrichment of genetic variability giving rise to variations/mutations at an unexpectedly high rate and may be a novel source of genetic variability in many plant species (Scowcroft *et al.,* 1987). The power of *in vitro* cell line selection approach shall be screening independent of environment. Efficient callus induction shall be the fundamental step in *in vitro* cell line selection. In fact, Shashidhara (2005) and Lokesha *et al*., (2005) have standardized callus induction protocol in sesame through direct seeding method. Using this protocol, callus induction was possible in all susceptible and resistant genotypes in the absence of toxin. However, when callus induction was attempted on MS media with pathotoxin of *Alternaria sesami*, calli of susceptible genotypes could survive only up to 300 ppm culture filtrate load (Figure 3) whilst that of resistant genotype could not only survived but also proliferated up to 700 ppm dosage (Fig 4; Figure 5 – 5a & 5b). This indicates the true resistance nature of RT-273 genotype, identified through field screening, callus against *Alternaria sesamii* pathotoxin. Exposure to six hours of UV radiations practically killed the entire callus. But the best survival was with one hour. Hence, one hour was taken as the criteria for in vitro mutagenesis. Intriguingly somaclones were induced in susceptible genotypes with 1000 ppm load (3.33 times more toxin) of toxin where un-irradiated callus failed to survive at 300 ppm load. This clearly indicates the creation of new variation at callus/cell level. In fact, desirable morphological traits such as disease, insect and acid resistance and even salt tolerance somaclones have been generated by a few workers in other crops (See Shawn *et al*., 2000).

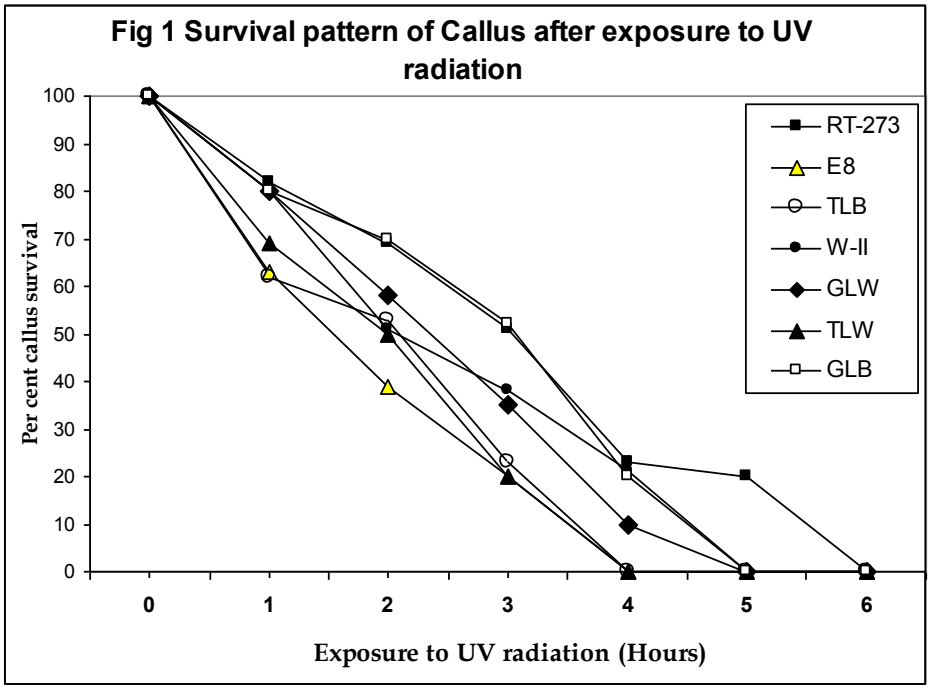
Table 1. Salient features of Sesame genotypes used in the study.

Genotype/ variety	Reaction to Alternaria blight disease	Seed coat color	Seed Source	Remarks
E8	Susceptible	white	Maintained by primary author	Nationally released variety
Gulbarga local white (GLW)	Susceptible	white	Collection from farmers field from Gulbarga region of Karnataka	Land race, Late maturing
Gulbarga local Brown (GLB)	Susceptible	brown		Land race, Late maturing and powdery mildew resistant
Western II (W II)	Susceptible	white	ARS, Mandore, Rajasthan	Released variety for Rajasthan, early maturing, moderately tolerant to Phyllody
Tumkur Local Brown (TLB)	Susceptible	brown	Collection from farmers field from Tumkur region of Karnataka	Land race, Late maturing and powdery mildew resistant
Tumkur Local White (TLW)	Susceptible	white		
RT 273	Resistant	Brown	ARS, Mandore, Rajasthan	A genotype from a local collection of Mandore region

Table 2. Somaclones generated with 1000 ppm partially purified toxin concentration and one hour exposure of different susceptible genotypes of sesame.

Sesame genotype/variety		No. of Callus inoculated	No. of Somaclones generated	Total number of Somaclones survived after sub-culturing
Gulbarga Local	Black	18	08 (44.44)	6 (75.00)
	White	19	02 (10.53)	-
Tumkur Local	Black	09	03 (33.33)	-
	White	15	01 (0.07)	-
E8		20	17 (85.00)	9 (53.94)
W-II		09	02 (22.22)	-
Total		90	33 (36.67)	15 (45.45)

(Values in parenthesis are percentages)



Pure Culture of *A. sesami* (from a single spore) +
Czapeck's Agar Medium
↓
1 Cm dia disc of fungus
↓
Czapeck's Broth Media
(50 ml in 250 ml Conical Flask)
↓ **(Incubation for 11 days)**
Filtration through Cheese cloth and Whatman Number 42
↓
Centrifuged at 12000 rpm for 10 min
↓
Supernatant was reduced to 1/5th of its original volume
by evaporating at 46º C in hot water bath
↓
Add 2 volumes of Acetone
↓
Constant stirring till precipitation – stored at 4º C
↓
Precipitation was removed by centrifugation at 20000 rpm for 10 min
↓
Hot water bath at 40º C (to remove Acetone)
↓
Adjusted the solution to original volume (viz. 1/5th) with water
↓
3 time extract with 2 parts of aliquots of water saturated 1-Butanal
↓
Water phase discarded and Butanal phase was combined and kept on
Hot water bath at 40º C till complete dryness
↓
Residual of Butanal + 100 ml water extracted 3 times
with 200 ml of aliquots of water saturated Butanal
↓
Water phase was again discarded and the Butanal phase was combined and dried
↓
The dried residues were dissolved in 200 ml of water.
↓
It was extracted with 400 ml of diethyl ether
↓
Ether phase was discarded
↓
Water phase was taken in to dryness by hot water bath and dried
Product was pathotoxin. It was removed as crystalline powder
↓
Stored in -20º C deep freezer

Figure 2. Isolation of *Alternaria sesami* pathotoxin protocol

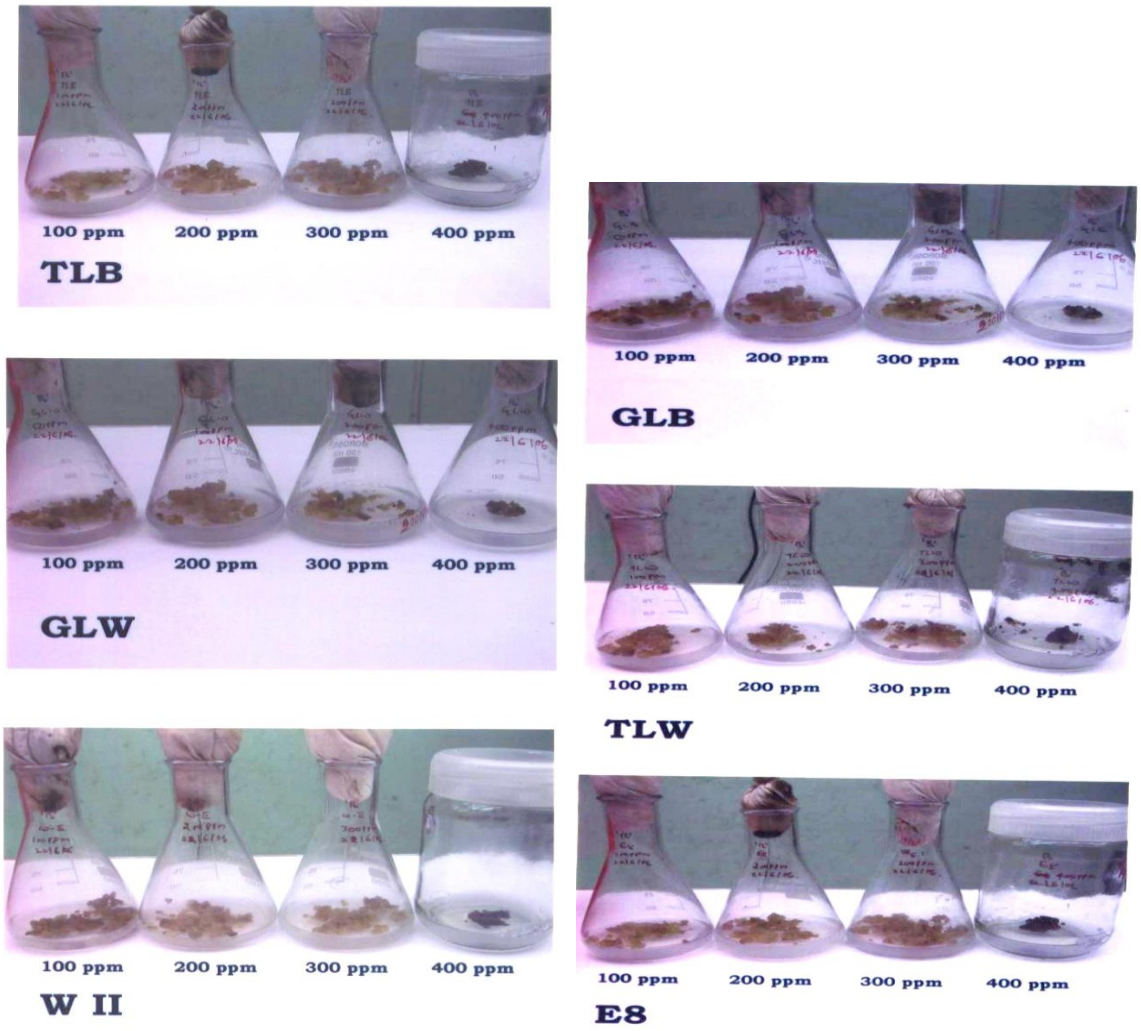


Figure 3. Callus survival of susceptible sesame genotypes across pathotoxin concentration.

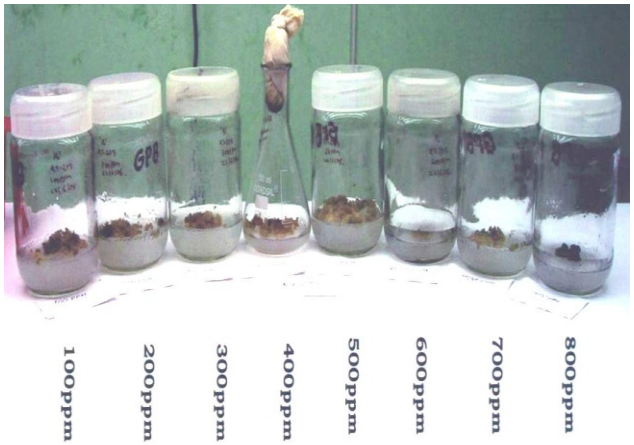


Figure 4 Callus survival *vs* toxin concentration of RT 273 génotype.

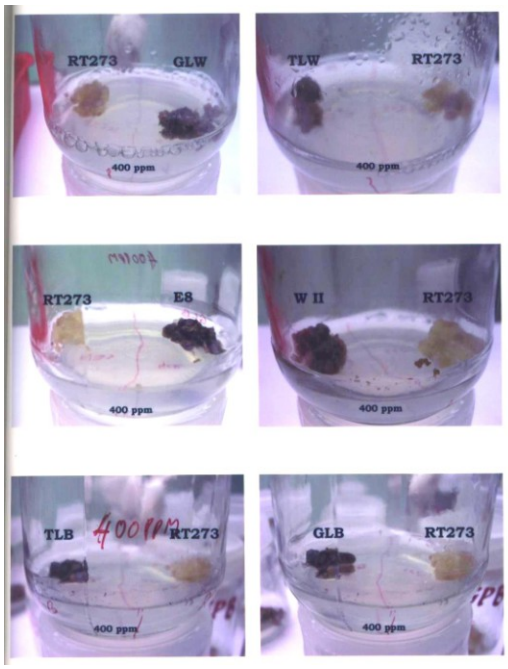


Figure 5a. Survival of callus of susceptible and resistant genotypes one week after inoculation at 400 ppm pathotoxin concentration.

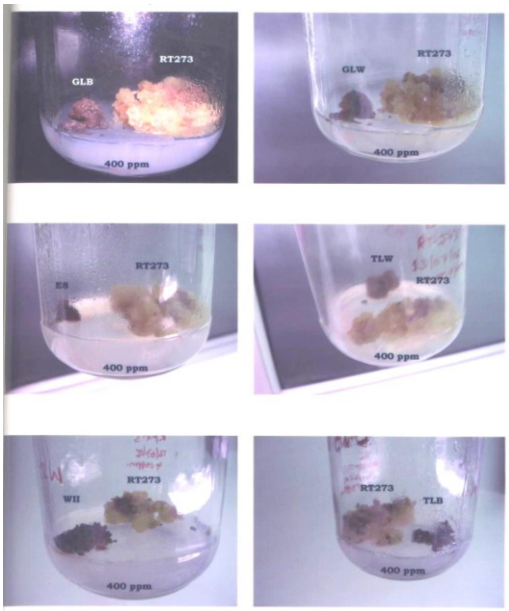


Figure 5b. Proliferation of callus of resistant genotypes four weeks after inoculation at 400 ppm pathotoxin concentration

One of the major bottlenecks in tissue culture of sesame is shoot regeneration. Indeed, several workers have attempted to regenerate through callus but with very low success. Interestingly, the concerted attempts by Shashidhara (2005) have achieved success of regeneration to a notable extent that could increase up to 100 per cent (Bangaramma, 2009). *Alternaria* blight has been identified as a major disease of Sesame that limits seed yield both qualitatively and quantitatively (Lokesha *et al.,* 2005). E-8 is a white seeded very popular variety of national importance; hence induction of somaclones in E-8 that are resistant or tolerant to Alternaria blight shall be very useful. Sub-culturing of callus for maintenance and regeneration is another important step in tissue culture. In fact, a good number of calli that were resistant to higher levels of pathotoxin have been successfully sub-cultured. It will be interesting to regenerate from resistant callus and to evaluate regenerated plants and test them under natural field conditions to achieve practically acceptable resistant genotypes that can be released for cultivation.

REFERENCES

Ashoka J (2001). Regeneration and in vitro selection cell lines resistant to stem and pod rots in Groundnut (*Arachis hypogeae* L.). *M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad.*

Bangaramma, SW (2009). Studies on standardization of protocol for high frequency regeneration in Sesame (*Sesamum indicum* L.). *M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Raichur.*

Chae, YA, Park SK and Anand IJ (1987). Selection *in vitro* for herbicide tolerant cell lines of *Sesamum indicum* L. 2. Selection of herbicide tolerant calli and plant regeneration. *Korean Journal of Beeding* **19** 75-80.

Janagoudar BS (2000). *In vitro* salt tolerance and protoplast studies in cotton (*Gossipium herbaceum* L.). Post Doctoral Report Submitted to the Plant Science Division, School of Biosciences, Nottingham, UK.

Kariyallappa DK (2003). *In vitro* regeneration and cell line selection for *Alternaria* resistant callus in Sesame (*Sesamum indicum* L.). *M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad.*

Kim MK, Park SK and Chae YA (1987). Selection *in vitro* for herbicide tolerant cell lines of *Sesamum indicum*: Effects of explants and hormone combination on callus induction. *Korean Journal of Plant Breeding* **19** 70-94.

Larkin PJ, Ryan SA, Breettel RJS and Scowcroft WR (1984). Heritable somaclonal variation in wheat. *Theoretical and Applied Genetics*, **67**: 447-455.

Lokesha R, Shashidhara N and Janagoudar BS (2005). A new and rapid method of successful callusing in sesame (*Sesamum indicum* L.) through direct seeding. *Plant Cell Biotechnology and Molecular Biology,* **8**(1&2) 85-88.

Ram R, D Catlin, J Romero and C Cowley (1990) Sesame: New approaches for crop improvement. In: J. Janick and J.E. Simon (Eds.), Advances in New Crops. Timber Press, Portland, OR. Pp: 225-228.

Scowcroft, WR, Brettell RIS, Ryan SA, Davies PA, Pallotta MA (1987). Somaclonal variation and genomic flux. *Plant Tissue and Cell Culture*, *Alan R. Liss. Inc*. 275-286.

Shashidhara N (2005), Shoot regeneration *via* rapid method of callusing in sesame (*Sesamum indicum* L.). *M.Sc. Thesis*, *Uni. Agric. Sci. Dharwad.*

Shawn MK, HF Kaeppler and Y Rhee (2000). Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology* **43** 179-188.

Venkatachalam P and Jayabalan N (1996). Efficient callus induction and plant regeneration via somatic embryogenesis, from immature leaf derived properties of Groundnut (*Arachis hypogeae* L.). *Isralean Journal of Plant Sciences* **44** 387-396.

Venkatachalam P, Shanker Rao K, Kishore PRK and Jayabalan N (1998). Regeneration of late leaf spot resistant groundnut plants from *Cercospora personatum* culture filtrate treated callus. *Current Science* **74** 61-65.