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RESEARCH ARTICLE

Adventitious shoot regeneration in Sesame (*Sesamum indicum* L.) (Pedaliaceae) *via* deembryonated cotyledonary explants

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ABSTRACT

Adventitious shoot regeneration in sesame, *via* de-embryonated cotyledonary explants, was attempted using six diverse genotypes; two were land races (Kankapura Local – KNL and Tamil Nadu Local- TNL), three were released varieties (DS-1, DSS-9 and WII) and a breeding line (RT-273). Three media compositions (½ MS basal media supplemented with 20 µM TDZ + 2.5 µM IAA and with or without 25 µM BAP and full MS basal media supplemented with only 25 µM BAP) were used of which highest shoot regeneration (100%) with 8.15 and 7.37 shoot numbers per cotyledon was achieved in RT-273 in both the combinations (½ MS with 20 µM TDZ+ 2.5 µM IAA+ 25 µM BAP or without BAP) followed by DS-1(100% and 95.55% with 6.15 and 3.89), KNL (88.88% and 7.55) and TNL (88.88% and 5.73) respectively in ½ MS with 20 µM TDZ+ 2.5 µM IAA+ 25 µM BAP. MS with 25 µM BAP alone failed to induce shoot regeneration.

Key Words: Adventitious shoot regeneration, de-embryonated cotyledons, MS media, Sesame, *Sesamum indicum*.
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INTRODUCTION

Sesame (*Sesamum indicum* L.), a member of Pedaliaceae family, is an important and oldest oil seed crops known to man and was an integral part of ancient legends, has early origins in East Africa and India as well (Nayar and Mehra, 1970; Bediginan and Harlon, 1986). Indeed, it was in cultivation in India as well (Joshi, 1961) mainly for edible oil and privileged as "Queen of oil seed crops" (Weiss, 1971). Conventional breeding methods are time consuming and sometime not possible (Sardana, 1998). Advances in tissue culture techniques offer an immense promise for sesame improvement. Shoot regeneration from hypocotyls and/or cotyledonary explants have been reported by several workers but at very low frequencies (Rao and Vaidyanath, 1997; Taskin and Turgut, 1997; Kim, 2001; Kariyallappa, 2003; Shashidhara*et al.*, 2008; Were *et al.*, 2006; Seo et al., 2007; Bangaramma, 2009 and Manju*et al.*, 2010). In fact, with several failures, high frequency regeneration was achieved through hypocotyls (Bangaramma and Lokesha, 2010). But, a dependable and reproducible regeneration protocol, across varieties that are of local importance, is still a bottleneck in sesame. Intriguingly, shoot regeneration is a vital step in tissue culture for genetic transformation and also for transfer of genes related to biotic and abiotic stresses. Creating somaclones using *in vitro* cell line selection for Alternaria blight fungal resistance in sesame has been attempted (Kariyallappa 2003; Praveen, 2006; Lokesha and Naik, 2011) and was successful in inducing desirable somoclonal variation. However, the regeneration was not possible owe to lack of a dependable protocol. Somatic embryos have also been induced from hypocotyl-derived calli, but no plant regeneration was achieved (Mary and Jayabalan, 1997; Bangaramma, 2009; Bangaramma and Lokesha, 2011). Somatic embryogenesis from hypocotyls segments (Mary and Jayabalan, 1997) and cotyledon and root and sub-apical hypocotyls segments from younger seedlings (Zeevaart and Creelman, 1988) of *Sesamum indicum* L. has been reported. However, Xu *et al.* (1997) reported that plant conversion rate from somatic embryos was very low (less than 12 to 13%).

For the first time, high regeneration ability of de-embryonated cotyledon explants *via* adventitious shoot formation was reported by Seo *et al.* (2007) in sesame globally, though it has already shown in other oil seed crops. It was adopted by Rahimansab (2011) from India and Shafeay *et al.* (2011) from Egypt and shown that adventitious shoot formation, with a high frequency, is possible. Influence of different age of de-embryonated cotyledons (0, 2, 4 and 6 days old) on regeneration ability in sesame was reported by Rahimansab (2011) and Lokesha *et al.* (2012) and they concluded that age of de-embryonated cotyledons and regeneration ability were inversely correlated; zero day old de-embryonated cotyledons were best suited than 2, 4 and 6 days old cotyledons. In the present investigation, an attempt was made to use the cotyledons of zero day old, as identified by Rahimansab (2011), extended to six varieties that were of local importance and can enhance genetic improvement process and possible opening of new avenues in genetic improvement of sesame crop (poor man's crop) both locally and globally.

MATERIALS AND METHODS

Plant materials:

Six genotypes/varieties of sesame (*Sesamum indicum* L.) of which two were land races (TNL and KNL), three were released varieties (DS-1 selection from local landrace from UAS Dharwad, DSS-9 a mutant variety released by UAS Dharwad, and W II from Gujarat) and one was a breeding line from Rajasthan (RT-273). Genotypes in this study were known for different characters (Table 1).

Surface sterilization:

Healthy and uniform seeds were soaked overnight in water and were washed by continuously running tap water for 15 minutes followed by sterile distilled water. Seeds were disinfected with 70% alcohol for 2 min and then surface-sterilized

with 0.1% (w/v) aqueous solution of mercuric chloride for 5 min under laminar air flow cabinet. The seeds were subsequently washed four to five times with sterile double distilled water to remove traces of mercuric chloride.

Table 1. Salient features of sesame genotypes/varieties.

Sl. No.	Genotype/ variety	Reaction to Disease	Seed coat colour	Seed source	Remark
1	RT-273	Resistant to Alternaria blight	Brown	ARS, Mandore, Rajasthan	A genotype from a local collection of Mandore region
2	Tamil Nadu Local (TNL)	Resistant to Phyllody	Brown	A collection from Tamil Nadu	Land race
3	Kanakapura Local(KNL)	Resistant to powdery mildew	Brown	Local collection from Kanakapura area of southern Karnataka	Land race
4	DSS-9	Susceptible to Phyllody, and powdery mildew but resistant to *Cercospora* leaf spot	White bold seed	UAS, Dharwad	Released variety from UAS, Dharwad through mutation breeding
5	DS-1 (Dharwad Selection-1)	Susceptible to *Alternaria*,Phyllody and powdery mildew but resistant to *Cerospora* leaf spot	White	Procured from Sesame breeder, UAS, Dharwad	Selection from local variety and released variety from UAS Dharwad
6	W-II (WesternII)	Moderately tolerant to Alternaria blight and Phyllody	White	Open market supplied from Gujarat available in one Kg packet	Released variety from Gujarat

Preparation of explants:

With the help of stereo binocular microscope, embryos were excised from seeds by removing seed coat followed by aileron layer then longitudinal cut was given to separate two intact cotyledons attached by embryonic axis (de-embryonated cotyledons) as suggested by Seo *et al.* (2007) and adapted by Rahimansab (2011). These explants were inoculated on to culture medium, for regeneration with and without embryonic axis of cotyledons (Fig. 1).

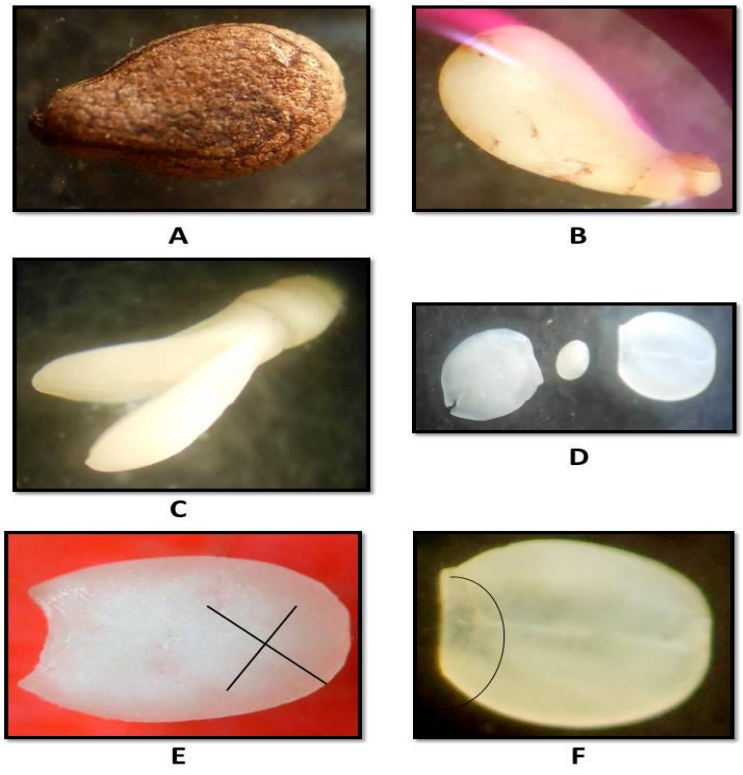

Fig 1. De-embryonated cotyledon of *Sesamum inducum* L. as explant. A - Over night soaked seed of *Sesamum inducum* L.; B - Seed removed with seed coat; C - Two cotyledons intact with embryo and embryonic axis; D - Seperated cotyledons from embryo with embryonic axis; E - De-embryonated cotyledon with intact embryonic axis; F - De-embryonated cotyledon with out embryonic axis

Effects of plant growth regulators on regeneration ability and multiple shoot induction:

De-embryonated cotyledon explants were inoculated in culture bottles containing three combinations *viz*. ½ MS basal media supplemented with 20 µM TDZ + 2.5 µM IAA and with or without 25 µM BAP and MS basal media with 25 µM BAP alone. Five cotyledons were inoculated in to each bottle and replicated three times for each concentration. All the bottles were incubated in dark for 24 h before they were shifted to light (1000 lux) and maintained up to six weeks for shoot regeneration. The culture room was maintained at 26±2°C with moderate relative humidity 80-85% (Shashidhara, 2005 and Lokesha *et al*., 2007).

RESULTS AND DISCUSSION

Shoot regeneration (100%) with 8.15 and 7.37 shoot number per cotyledon was achieved in RT-273 (figure 2) followed by DS-1(100% and 95.55% with 6.15 and 3.89 shoots) in both the combinations (½ MS with 20 µM TDZ+ 2.5 µM IAA+ 25 µM BAP (Table 2) or without BAP (Table 3). KNL (88.88% and 84.44% with 7.55 and 7.22 shoots) and TNL (88.88% with 5.73 and 4.84 shoots) recorded higher regeneration compared to DSS-9 which recorded moderate regeneration (73.3% and 71.11% with 2.60 mean shoot numbers per cotyledons). W-II recorded least regeneration (42.00% and 37.77% with 1.35 and 1.11 mean shoot numbers per cotyledon). Full MS media with 25 µM BAP failed to induce any shoots across varieties however, only 6.66% and 2.22% regeneration with one shoot only was noticed in DSS-9 and KNL respectively. Undeniably, ½ MS media with 20 µM TDZ+ 2.5 µM IAA with or without BAP had almost same mean shoot regeneration (~80%) with similar mean shoot number with more or less same spectrum of variation (Fig 3; Table 2 and 3) ('t' test is NS).

Fig 2.Shoot regenerability of RT273 on ½ MS + 20 µM TDZ + 2.5 µM IAA with or without BAP

Fig 3.Multiple shoots regeneration on ½ MS + 20 µM TDZ + 2.5 µM IAA + 25 µM BAP. RT-273 (left) and DS-1 (right)

Table 2. Shoot regeneration from de-embryonated cotyledonary explants placed on ½ MS with 20 µM TDZ+ 2.5 µM IAA+ 25 µM BAP.

Varieties	No. of cotyledons inoculated	No. of cotyledons responded	Shoot Regeneration (%)	Mean No. of shoots/cotyledons
RT-273	45	45	100	8.15
TNL	45	40	88.88	5.73
KNL	45	40	88.88	7.55
DSS-9	45	33	73.33	2.60
DS-1	45	43	95.55	6.15
W-II	45	19	42.00	1.35
Mean ± SD	45	36.67 ± 9.56	81.44 ± 18.720	5.25 ± 2.72

Table 3. Shoot regeneration from de-embryonated cotyledonary explants placed on ½ MS 20 µM TDZ+ 2.5 µM IAA without BAP.

Varieties	No. of cotyledons inoculated	No. of cotyledons responded	Shoot regeneration %	Mean No. of shoots/cotyledons
RT-273	45	45	100.00	7.37
TNL	45	40	88.88	4.84
KNL	45	38	84.44	7.22
DSS-9	45	32	71.11	2.60
DS-1	45	45	100.00	3.89
W-II	45	17	37.77	1.11
Mean ± SD	45	36.17 ± 10.57	80.36 ± 19.792	4.50 ± 2.50

De-embryonated cotyledons, single or sliced or both, have been used in inducing shoots in oil seed crops (more precisely groundnut) owe to high regeneration ability *vis a vis* sesame (Seo *et al*., 2007; Lokesha *et al*., 2012 and Shafeay *et al*., 2011). A large number of shoots have been produced through cotyledonary explants; Rahimansab (2011) had observed almost 8.50 shoots per cotyledons in sesame. In fact, in the present investigation, the shoot number was more or less same to that of Rahimansab (2011). Three genotypes (DS-1, RT-273 and WII) used by Rahimansab (2011) were common in the present investigation. But W-II had highest regeneration ability according to Rahimansab (2011), whilst it had least in the present investigation. This could be because of change in seed source as W-II is a variety that comes to market in packed form from Gujarat.

Genotype, growth regulators and type of explants have been shown to be critical factors for somatic embryogenesis and shoot organogenesis in crop plants (Venkatachalum *et al*., 1999). Some reports revealed that the age of explants is one important factor for shoot regeneration ability. It was possible to achieve shoot regeneration from zero day (Were *et al*., 2006; Seo *et al*., 2007 and Rahimansab, 2011), 2-4 days (Were *et al*., 2006 and Rahimansab, 2011), 6-10 days (George *et al*., 1987; Rahimansab, 2011 and Lokesha *et al*., 2012) and 1-2 weeks (Seo *et al*., 2007) old cotyledons. Whereas regeneration from cotyledons obtained from germinated seeds drastically declined (Were *et al*., 2006; Seo *et al*., 2007 and Rahimansab, 2011) and zero day old cotyledons (from mature seeds) found best suited compared to 2, 4, 6 days old cotyledons in sesame (Rahimansab, 2011). Intriguingly, zero day old cotyledons were used in the present investigation considering the experiences of Rahimansab (2011). Genotypic variability in shoot regeneration response has been reported by earlier workers (Kariyallappa, 2003; Shashidhar, 2005; Were *et al*., 2006; Seo *et al*., 2007; Bangaramma, 2009; Rahimansab, 2011; Shashidhara *et al*., 2011 and Shafeay *et al*., 2011) and may be attributed to genetic makeup with endogenous levels of auxins and cytokinins concerned with the embryonic phase of development (Chawla, 2002).

The media composition followed is adapted from Were *et al.* (2006); Rahimansab (2011) and Lokesha *et al*. (2012) Addition of 25µM BAP to ½ MS 20 µM TDZ+ 2.5 µM IAA combination has not changed the trend in regeneration ability of different genotypes however; it has improved positively in KNL, DSS-9 and W-II whilst it had a negative effect in DS-1. These findings were in accordance with earlier workers (Devi *et al*., 1994; Baskaran and Jayabalan, 2005 and 2006; Were *et al*., 2006). BAP is considered to be an effective cytokinin for stimulating shoot induction in combination with auxins at different concentrations. Both cytokinins (BAP and TDZ) were inferior when used alone but showed a good response for shoot regeneration when combined (Shashidhara *et al*., 2011).

No regeneration (0%) in most of the varieties tried (RT-273, TNL, DS-1 and W II) was observed when 25 µM BAP alone was used in full strength MS basal media. But two genotypes (DSS-9 and KNL could produce one shoot with a very low frequency of shoot regeneration (2.22 and 6.66% respectively). Shafeay *et al*. (2011) also observed similar results, either they could observe callus or shoot primordial formation from de-embryonated cotyledons on MS media supplemented with different levels of BAP (0.00, 2.00 and 4.00mg/l) alone. Cytokinin alone caused browning and subsequent death of the explants (Were *et al.*, 2006), which was observed in present investigation also when de-embryonated cotyledons inoculated on full strength MS media containing 25 µM BAP alone as growth hormone (Fig 4). Usage of TDZ in sesame tissue culture is very scanty (Were *et al.*, 2006; Seo *et al*., 2007; Shashidhara *et al.*, 2011 and Rahimansab, 2011) though it has been found to be a better cytokinin. Shoot regeneration probably depends on the levels of endogenous growth regulators and TDZ modulates endogenous auxin levels (Murthy *et al.*, 1995 and Hutchinson and Saxena, 1996).

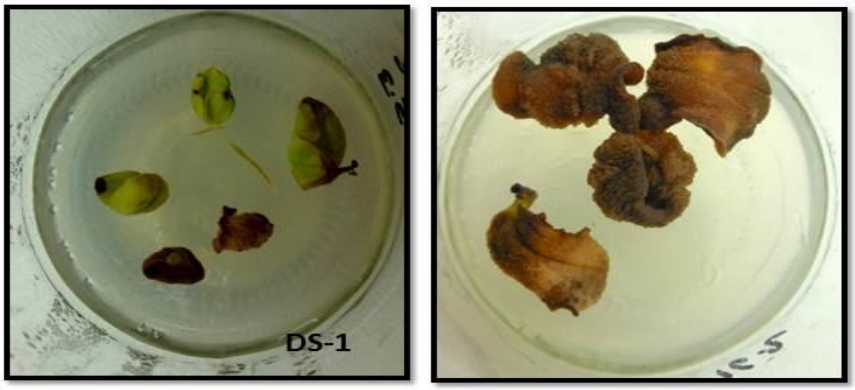

Figure 4.Browning and death of explant on full strength MS + 25 µM BAP alone.

BAP reduces number of shoots per explants while TDZ ensured the health of the cultures unlike BAP that often causes browning. TDZ and IAA were superior to BAP which has been observed by Were *et al.* (2006). Replacement of TDZ with BAP significantly reduced the regeneration frequency.

Loss of embryonic axis could be fatal while excising cotyledons from seeds after soaking which could both crucial and skilful step. However retaining of embryonic axis has not seen or clearly mentioned by earlier workers (Seo *et al*., 2007; Shafeay *et al*., 2011and Lokesha *et al*., 2012). In the present investigation embryonic axis retention was compulsory.

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