Influence of Plant Growth Regulators and Explant Type on Multiple Shoot Induction and Somatic Embryogenesis in Sesame (*Sesamum indicum L.*)

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Abstract— *Sesamum indicum L. is used as an important oil crop in the world. For establishing of a simple and a rapid system for in vitro culture of sesame shoot tips and hypocotyls explants were cultured on MS media with differtent combinations and concentrations of PGRs. On medium with 5 mgl^{-1}BA plus 1 mgl^{-1} IAA and 1 mgl-^{1} ABA mutliple shoot induction on explants was occurred. Multiple shoot indution on medium supplemented with 2 mgl^{-1}BA and 0.3 mgl^{-1} NAA happen with a short phase of callusing. Also MS medium with 3 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin was a suitable medium for inducing of somatic embryogenic calli with the frequency of sub-culturing potential. Regenerated shoots on both media rooted on MS medium plus 1.5 mgl^{-1} NAA and 0.03 mgl^{-1} BA and whole regenerated plantlets formed.*

Abbreviations*: 2,4-D – 2,4-dichlorophenoxy acetic acid; ABA – Abscisic acid; BA – 6 benzylaminopurine; IAA – Indole – 3 – acetic acid; IBA – Indole – 3 – butyric acid; Kinetin – n^{6} – Furfyrylaminopurine; MS – Murashige and Skoog medium; NAA – Naphtalene acetic acid; PGRs – Plants Growth Regulators.*

Key words: adventitious shoots, embryogenic calli, sesame.

I. INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important crop in tropical and subtropical areas. This important crop ranks third among the oilseed in production. Its oil content varies from 44 – 66 % containing two unsaturated fatty acids – oleic and linoleic together account for 85% (Maximum) with a combination of different essential amino acids and vitamins particularly β carotene (Brar, 1982 and Arslan *et al*., 2007). Sesame cultivated in Iran in central, north – western, north – eastern, western and eastern parts. Also sesame is one of the most desirable oils due to demanded perfume and demanded flavour in our country. Cultivation of sesame suffers from considerable yield loss because of pathogenic diseases like phytophthora blight and root/stem rot (Gangopadhyay *et al*., 1998). In addition, it is difficult to the time of harvest a sesame crop to maximize yield because plant growth is indeterminate and capsules dehisce when mature (Day, 2000). Conventional methods for improvement is slow, felt difficult, owing to nonavailability of desired genetic variation in cultivated types. Though a few wild species are known to carry resistance genes, it is difficult to transfer owing to presence of inter-specific barriers (Shashidhara *et al*., 2011).

Biotechnological approach like exploitation through somaclones, genetic transformation requires a dependable, flexible and reproducible callus initiation and shoots regeneration system (Shashidhara *et al*., 2011). So in the present investigation, we made an attempt to use different plant growth regulators and different explants for callus induction and producing somatic embryo and regenerated organs (shoot and root) in sesame.

II. MATERIALS AND METHODS

Seeds of sesame CV. Darab1 were obtained from Seed and Plant Improvement Institute, Karaj, Iran.

Mature seeds of *S. indicum* L. were aseptically sterilized by immersing in 100% commercial hypochlorite sodium (with 5% available chlorine) for 30 min. After washing with sterilized distilled water for 4 – 5 times, the seeds were cultured on half – strenght Murashige and skoog (MS) (Murashige and Skoog, 1962) medium, MS medium, half – strenght Gamborg medium (B_5) (Gamborg, 1968) and B_5 medium with 30 g l^{-1} sucrose and 7.5 g l^{-1} bacto agar. Hypocotyl (4 – 5 mm) and shoot tip (2 – 3 mm) explants from 1 week – old seedlings cultured on MS medium including different kind and concentrations of PGRs (IBA, BA, NAA, KIN, IAA, ABA, 2,4-D) and with 100 mg l^{-1} *m*-inositol, 0.1 mg l-^{1} thiamine, 0.5 mg l-^{1} pyridoxine, 0.5 mg l-^{1} nicotinic acid, 2 mg l-^{1} glycine, 30 g l^{-1} sucrose and 4 g l-^{1} phytogel. All media adjusted to pH 5.8 before autoclaving at 120°C for 15 min. The culture room was maintained at 24 ± 1°C with a 16-h photoperiod and 50 µmol m^{-2} s^{-1} using cool – white fluorescent tubes. 10 hypocotyl explants and 5 shoot tip explants were cultured into 10-cm petri dishes

per treatment and the experiment was repeated 5 times. After about 1 month the induction of adventitious shoots and embryogenic calli were considered.

III. RESULTS AND DISCUSSION

3.1 Effect of basal media on growth and development of seedlings

Seed germination is an important steps of establishing plant tissue culture, because quality of growth and development of seedlings influence to have a uniform and suitable set of seedlings to the treatments (Sakhanokho *et al*., 2001). Among of different basal media that were evaluated for their effects on seed germination, half strength MS was the best medium based on longitudinal and trabsversal growth of seedlings (data were not shown) and rate of germination. That's a thought different between basal media are depended their salt concentrations. As mentioned earlier the half strength MS medium was the best medium for sesame seed germination. Our results agree with results of Droste *et al*., 2005 and Abdellatef and Khalafalla, 2007 that stressed in low salt-concentration media the highest in vitro germination rate was performed. Because in half strength MS medium the concentrations of salt are half.

3.2 Effect of PGRs and shoot tip explants on adventitious shoot formation

The shoot tip explants from 7 – days – old seedlings were cultured on MS medium with various kinds and concentrations of PGRs, alone or combination with others (Table 1). The best combination of PGRs for the highest rate of adventitious shoot formation was 5 mgl^{-1} BA plus 1 mgl^{-1} IAA and 1 mgl^{-1} ABA (Fig. A1). Also on medium with 2 mgl^{-1}BA and 0.3 mgl^{-1} NAA either rate or the numerous adventitious shoot per explants was considerable (Fig. A3).

Other media such as the combination of BA (0.1 mgl^{-1}) and IAA (1 mgl^{-1}) and that were pointed in table 1 was significantly less responsive for adventitious shoot formation (Fig. A4).

The most important characteristic of these two media is high level of BA. The positive effect of BA on the induction of regenerated shoot is clear. For example the positive effect of BA on shooting in *Cucurbita maxima* Duch (Lee *el al*., 2003), *Ruta graveolunse* L. (Ahmad, N. *et al*., 2010), tomato (*Lycopersicon esculentum* L.) (Rai *et al*., 2012) and Japanese pear (Kadota *et al*. 2001).

On medium with 2 mgl^{-1}BA and 0.3 mgl^{-1} NAA hypocotyls segments produced callus at low frequency, then produced adventitious shoots (as could be seen in Fig. A2). But on medium with 5 mgl^{-1}BA plus 1 mgl^{-1} IAA and 1 mgl^{-1} ABA callusing phase was very short and explants produced adventitious shoots directly. So the results of present study provide a rapid and proper system which could be used for either somaclonal variation induction or direct induction of organs and elimination effects of somaclonal variation.

TABLE 1
EFFECT OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF PGRS ON ADVENTITIOUS SHOOT FORMATION FROM SHOOT TIP OF 7 – DAYS - OLD SEEDLINGS IN *S. INDICUM L.*

IAA mgl^{-1}	BA mgl^{-1}	ABA mgl^{-1}	NAA mgl^{-1}	Frequency of shoot formation (%)	The mean No. of shoots/explants
1	0.1	1	0	30	2
1	1	1	0	30	2
1	2	1	0	27	2
1	3	1	0	45	3
1	4	1	0	_	2
1	5	1	0	98	15
0	0.1	0	0	12	3
0	1	0	0.1	12	3
0	2	0	0.2	19	2
0	2	0	0.3	95	11

3.3 Effect of PGRs and hypocotyls explants on somatic embryogenesis

Hypocotyl explants from 7 – days – old sesame seedlings cultured on MS media supplemented with different concentrations and combinations of PGRs. 2, 4-D plus kinetin was the best combination for highest rate of callusing (with somatic embryogenesis characteristic calli) (Table 2.). According to researchers hypocotyls are considered as model explant for callus induction (Shashidhara *et al*., 2011). Also in present study hypocotyls explants induced calli on media nearly 20 – 30 days after establishing. In fact 5 – 7 days after establishing of explants on media the cut surfaces of them became swollen then the callusing was begun slowly.

It is clear that initiation of embryogenic cultures start by culturing the primary explant on medium supplemented with PGRs, mainly auxin but often also cytokinin (Von alnold *et al*., 2002). In our study 3 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin was the best combination of PGRs for inducing the embryogenic calli in sesame (Fig. A6). Also about the role of 2,4-D in somatic embryogenesis scientists believed that the use of 2,4-D alone or in combination with other hormones has become almost routine in somatic embryogenesis. (Huang and Yeoman, 1984; Mordhorst *et al*., 2002). All of these declarations agree with our results.

TABLE 2
EFFECT OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF PGRS ON INDUCTION OF SOMATIC EMBRYOGEN CALLI FROM SHOOT TIP OF 7 – DAYS - OLD SEEDLINGS IN *S. INDICUM* L

2,4-D	BA	Kinetin	The mean of diameter of calli (mm)	Color of calli	The percentage of somatic embryogenic calli (%)
3	1	0	5	Brownish yellow	22
3	0	0.5	9	Brownish yellow	57
3	0	0	4.4	Brownish yellow	20
4	0	0	5	Brownish yellow	20

In addition to callus induction, callus maintenance is an important step also and must to be standardized (Shashidhara *et al*., 2011). General observation is that the callus turn brown after about more than five cycles of sub-culturing (Khanna, 1999). Whereas as could be seen in figures (Fig. A7 and A8) calli from above mentioed explants and above mentioned media have potential of numerous sub-culturing. These figures show the successful subculture of callus from above mentioned medium after 4 times and for a 2 month period.

3.4 Rooting and production of whole plantlets

Regenerated shoots were transferred to MS medium supplemented with kinds of auxins such as IAA, IBA, and NAA. NAA were used either alone or with 0.03 mgl^{-1} BA. Other auxins were added media from 1 – 1.5 mgl^{-1}. The results showed that the combination of NAA and BA (1.5 mgl^{-1} NAA and 0.03 mgl^{-1} BA) has the best effect on rooting the bases of regenerated shoots and formation of whole plantlets (Fig. A5). There are many reports that showed the combination of NAA and BA is the most effective combination for rooting in sesame (Chakraborti and Ghosh, 2010), (Raja and Jayabalan, 2011). Also there are reports about the positive effect of NAA alone on rooting of sesame (Kushwaha *et al*., 2010). So much is certain that the NAA is the best PGRs for In Vitro rooting in many of plants.

In conclusion we established a simple and rapid regeneration system in sesame via both direct and indirect adventitious shoots. Also we presented a system for callusing and sub-culturing of calli in this reculcitrant plant.

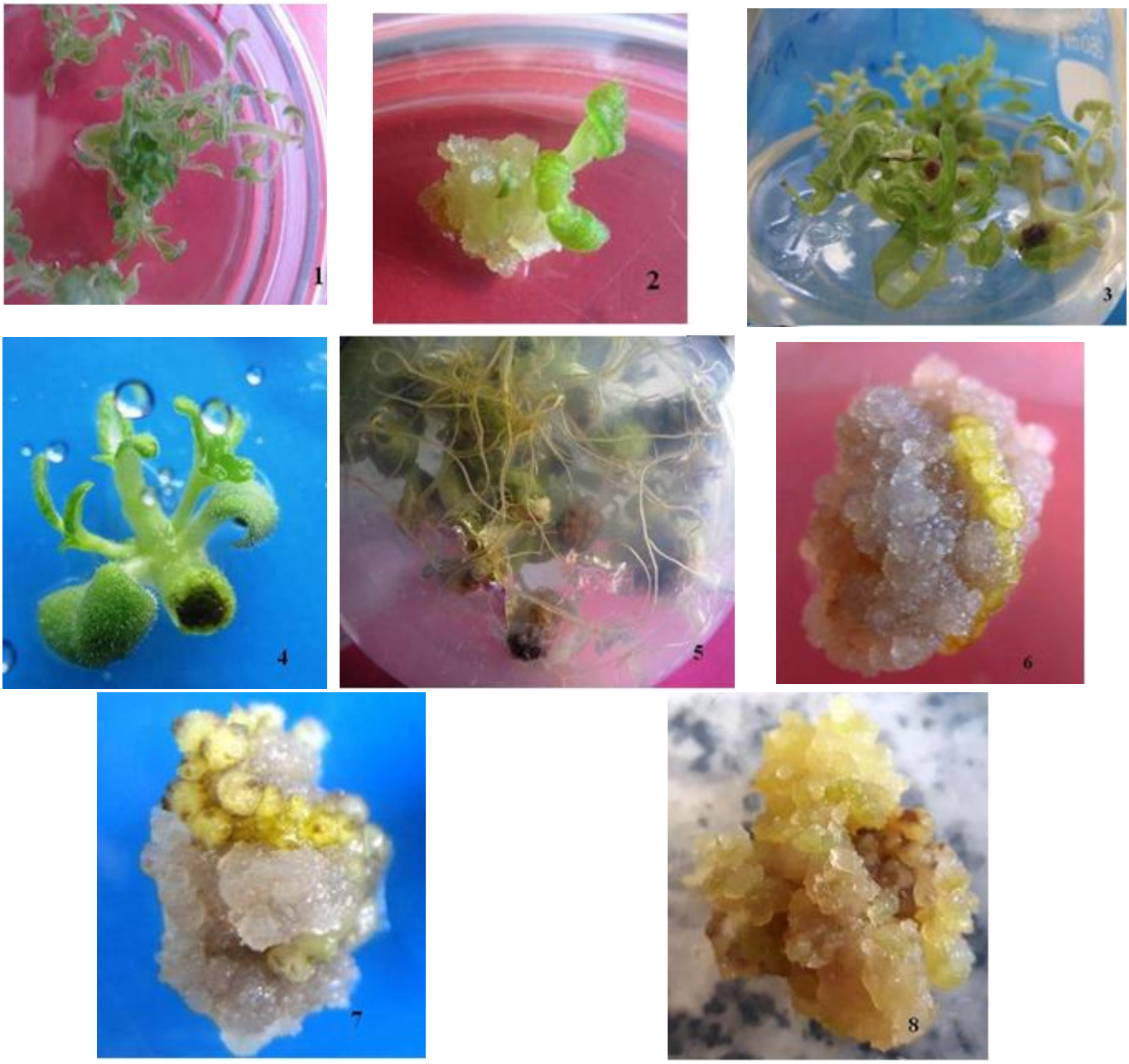

FIG . A ADVENTITIOUS SHOOTS AND SOMATIC EMBRYOGENIC CALLI FORMATION FROM SHOOT TIPS AND HYPOCOTYLS EXPLANTS IN 7 – DAYS – OLD SEEDLINGS OF SESAME. (1) DIRECT ADVENTITIOUS SHOOT ON MS MEDIUM WITH 5 mgl^{-1}BA plus 1 mgl^{-1} IAA and 1 mgl^{-1} ABA. (2) AND (3) INDIRECT ADVENTITIOUS SHOOT ON MS MEDIUM WITH 2 mgl^{-1}BA and 0.3 mgl^{-1} NAA. (4) SHOOT FORMATION FROM HYPOCOTYL EXPLANTS ON MS MEDIUM WITH 0.1 mgl^{-1} BA and 1 mgl^{-1} IAA. (5) ROOT FORMATION ON BASES OF ADVENTITIOUS SHOOT ON MS MEDIUM WITH 1.5 mgl^{-1} NAA AND 0.03 mgl^{-1} BA. (6) EMBRYOGENIC CALLI ON HYPOCOTYL EXPLANTS ON MS MEDIUM WITH 3 mgl^{-1} 2,4-D AND 0.5 mgl^{-1} KINETIN. (7) AND (8) EMBRYOGENIC CALLI ON HYPOCOTYL EXPLANTS ON MS MEDIUM WITH 3 mgl^{-1} 2,4-D and 0.5 mgl^{-1} KINETIN AFTER 4 TIMES AND FOR A 2 MONTH PERIOD

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