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In vitro Multiple Shoot Induction and Plant Regeneration in Elite Sudanese Sesame Cultivars (*Seasmum indicum* L)

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ABSTRACT

We report the first protocol for shoot induction and plant regeneration in elite Sudanese sesame cultivars (*Seasmum indicum*). Experiments were carried out using shoot tip explant from the cultivar Promo Ky. Later the optimized culture conditions were used to investigate the regeneration response of different genotypes. Shoot development was induced on Murashige and Skoog (MS) medium supplemented with Kinetin (KIN) or benzyladenine (BA) alone or in combination with α naphthalene acetic acid (NAA). BA proved to be more effective than KIN. The best response (2.7±0.1 shoot per explant), however was obtained when 1.0 mg L^{-1} BA was used. The efficiency of both BA and KIN for multiple shoot induction was negatively affected when combined with NAA. The isolated shoots were quickly rooted in the half-strength MS medium with or without growth regulators. However, the best result for rooting percentage (100%) and number of root per shoot (63.0 ±3.2) were obtained in presence of NAA at concentration of 0.05mg L^{-1} and well-rooted plantlets were successfully transferred to the soil following a standard hardening protocol. Genotypic differences were significant for both culture regeneration percentage and the number of shoots produced per regenerating explant. This protocol opens new biotechnological strategies to transfer economically important genes to this important crop species.

Key words: Sesame, Shoot induction, benzyladenine, hardening.

Introduction

Sesame (*Sesamum indicum* L.), family Pedaliaceae is one of the most ancient crops (Bedigian and Harlan, 1986). It is grown in tropical and subtropical areas (Ashri, 1998) on 6.5 million hectares worldwide, producing more than three million tons of seed (FAO, 2005). Sesame seed, is highly nutritive (50% oil and 25% protein), traditionally used for direct consumption and as a source of oil of excellent quality due to the presence of natural antioxidants such as sesamin and sesamol (Brar and Ahuja, 1979). Potentially beneficial effects of sesame on human health have recently renewed the interest in this ancient crop. Despite the nutritional value and historic and cultural importance of sesame, the research on sesame has been scarce (Laurentin and Karlovsky 2006).

In Sudan sesame is considered as one of the main cash crops. Sudan, India, Myanmar and China are the most important sesame producers with 68 % of the world production (Laurentin and Karlovsky 2006). In spite of the economical importance of sesame for the Sudan economy big fluctuations in production and yield occurred. This may be due to the cumulative effects of wide ranges of biotic and a biotic stresses such as plant diseases, indeterminate flowering, and seed shattering and lodging of plants. Conventional plant breeding methods have produced some improvement but have several limitations, such as access to a limited gene pool, crossing barriers, inefficient selection and being time consuming. Therefore, to overcome such problems of conventional breeding new technologies such as gene transfer which recently emerged as most important tool in agricultural research can be applied as alternative approach for genetic improvement of this crop.

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The potential benefits of using advanced agricultural biotechnology in sesame genetic improvement have not yet been realized in Sudan mainly, because the successful utilization of plant biotechnology for plant improvement requires the development of an efficient shoot regeneration system from cultured cells or tissues. The development of an efficient micropropagation protocol can highly support breeding of this potential and adaptive oil crop. Moreover, the establishment of cell culture has considerable potential to facilitate successful wide crosses using embryo culture techniques. Therefore there is an urgent need for developing an efficient *in vitro* regeneration protocol involving Sudanese sesame cultivars with regard to multiple shoots induction. Sesame in general, has proved to be very recalcitrant to regenerate *in vitro* (Were *et al*., 2006). However, Indirect adventitious shoot regeneration from shoot tip (Rao and Vaidyanath, 1997), nodal (Gangopadhyay *et al*., 1998) and leaf (Sharma and Pareek, 1998) cultures. hypocotyl and/or cotyledon explants (Rao and Vaidyanath, 1998b; Taskin and Turgut, 1997; Younghee, 2001) has been reported but at low frequencies. The present communication describes *in vitro* multiple shoot regeneration from shoot tip explants, and the rooting and successful greenhouse establishment of Sudanese sesame cultivars.

Material and Methods

Plant materials

Seeds of sesame cultivars used in this study were obtained from the Agricultural research and Technology Corporation (ARTC), Algdarief, Sudan

Surface sterilization and seed germination

Seeds were washed by continuously running tap water for 15 minutes then washed by sterile distilled water. Under laminar flow cabinet seeds were disinfected with clorex ® (0.5 % free chlorine) at concentration of 15% v/v for 15 mints then rinsed times with sterile distilled water. After surface sterilization, five seeds were directly transferred to culture bottle and incubated for 10 days at 25°C±2 with a 16 h photoperiod. Data on seed germination were recorded after 10 days of inoculation.

Effect of basal media on in vitro seed germination and explant

Four different basal media namely, full-salt strength MS (Murshing and skoog, 1962) medium, half-salt strength MS medium, Full -salt strength B5 (Gamborg *et al*., 1968) medium and half -salt strength B5 medium, were evaluated for their effects on *in vitro* germination of sesame seed. *In vitro* raised 14 days -old seedlings were used as source of shoot tip explants.

Effects of plant growth regulators and genotypes on multiple shoots Induction

Different concentrations and types of plant growth regulators were tested to assess their effects on multiple shoot induction. Shoot tip explant were cultured in culture bottles containing MS basal media supplemented with different levels (0.1, 0.5, 1.0, 1.5, 3.0 and 4.0 mg L^{-1}) of BA or Kn alone or in combination with NAA at 0.1 or 0.5 mg L^{-1} then incubated for four weeks at 25°c± 2 under 16 h photoperiod.

To assess the effect of genotype on multiple shoot induction, shoot tip explants obtained from ten sesame cultivars namely, Elgadareif-1, Elabassia, Promo-Ky, Aswad, Abusitta, Elobaied ,Khidir, Abusandog, Ali mahdi, Beladi) were cultured on MS media supplemented with 1.0 mg L^{-1} of BA and incubated for four weeks at 25°c± 2 under 16 h photoperiod.

Rooting of in vitro regenerated shoots and plantlet acclimatization

Shoots (2-3 cm) were excised and cultured on medium consisting of half strength MS basal medium supplemented with different concentrations (0.0, 0.025, 0.05, 0.5, and1.0 mg L^{-1}) of NAA, IAA or IBA. For acclimatization in *vitro* rooted shoots were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 3:1 ratio. Plants were kept under culture room conditions for 7 days then transferred to green house and placed under shade until growth was observed.

Culture condition and data analysis

All the media used in this study were supplemented with 3% (w/v) sucrose, solidified with 0.8% (w/v) and the pH was adjusted to 5.8 before addition of the agar and autoclaving at 121°C and 15 lb psi for 15 min. Results were observed at regular intervals and data were collected from three independent experiments and analyzed by using analysis of variance procedure (ANOVA) on excel computer program. Means were separated by Duncan's multiple range test (DMRT) (Duncan, 1955) and presented as average ± standard error (SE).

Result and discussion

Higher germination rate is an important factor for establishing plant tissue culture and be particularly useful when there is a need to submit a uniform set of seedlings to a treatment (Sakhanokho *et al*., 2001). Sesame *in vitro* seed germination was observed after 48h and within 3days produced well developed root system. Among the four different basal media evaluated for their effects on seed germination, half strength B5 basal media supported high rate of germination (93%) followed by half strength MS (83%), B5 (73%) and full MS (37%) (Table1). These variations in germination percentage between the basal media might be due to their basal salt formulation, and the high germination percentage obtained on half strength B5 media probably due to their low salt content compared to half strength MS, B5 and MS basal media. Droste *et al*., 2005 attributed the low *in vitro* germination rate of *Vriesea gigantean* and *Vriesea philippocoburgii* seeds to the sensitivity of the species to high salt-concentration present in MS medium. Moreover, in cotton the highest *in vitro* germination rate was reported in low salt-concentration media (Abdellatef and Khalafalla, 2007).

Table 1: The effects of different basal media on *in vitro* germination of sesame seed of promo Ky cultivar

Basal media	Germination % (mean ±SE)
MS	37±5.4
½ MS	83±5.5
B5	73±6.8
½ B5	93±3.2

The morphogenetic responses of shoot tip explants to BA and Kn either alone or in combination with NAA are summarized in (Table 2). Direct shoot bud differentiation was observed after 2 weeks of culture initiation. Multiple shoots were initiated from the shoot tip explants after 4 weeks of culture (Fig. 1A). The frequency of shoot formation was influenced by types and concentrations of growth regulators used. Explants obtained from two weeks old seedling of sesame cultivar promo Ky cultured on hormone-free MS basal medium failed to show any response but remained green up to four weeks. However, on MS basal medium supplemented with various concentrations of Kn or BA alone or in combination with NAA enlarged in their size after one to two weeks of culture and adventitious shoots developed directly in another four weeks.

BA at different concentrations induced more shoots per explant compared to Kn at the same concentration (Table 2) indicating that BA is more effective than Kn for multiple shoot induction in sesame. The beneficial effect of BA on multiple shoots induction has already been reported for sesame. (George *et al*., 1987, 1989; Rao and Vaidyanath1998a).Moreover, the efficiency of BA over Kn was agreed on in different plant regeneration systems (Bhojwani, 1980; WeLander *et al*., 1989; Devi *et al*., 1994; Baskaran and Jayabalan, 2003; Baskaran and Jayabalan, 2005).

The regeneration percentage and shoot regeneration frequency increased with increases in concentration of BA until it reaches 1.0 mg L^{-1} which was found to be the optimal concentration for maximum frequency of sesame shoot bud formation (2.7±0.1). However, at higher concentration the number of shoot per explant was reduced (Table 2). This is mainly, because at higher cytokinin level shoot tip explant produced excessive callus and failed to improve the efficiency of shoot multiplication. Thiem (2003) reported that callus growth on explant usually interfere with the propagation process.

The inclusion of NAA (0.1 and 0.5 mg L^{-1}) in combination with Kn or BA in the culture medium was not effective in enhancing shoot proliferation, but supported prolific callus growth at the basal end of explants. The inhibitory effect of NAA on multiple shoot induction has been demonstrated in numbers of plants, including faba bean (khalafalla and Hattori, 2000) mung bean (Gulati and Jaiwal, 1992) and cotton (Abdellatef and Khalafalla, 2007). In the present study cytokinin (BA) alone was found to be suitable for multiple shoot induction, this revealed that exogenous auxin (NAA) was not essential to initiate shoot bud formation. However, the addition of cytokinin promoted the development of more shoots, thereby demonstrating the requirement of exogenous cytokinins for multiple shoot induction. In consistent with this result, Vengadesan *et al.* (2002) have reported that when cotyledonary nodes explants of *Acacia sinuata* were cultured on MS medium containing a combination of BA and auxins (NAA, IBA and IAA), the number of shoots was reduced but in turn produced basal callus.

Table 2: Effect of plant growth regulators on shoot induction from shoot tip explant of Promo- Ky sesame cultivar after 6 weeks of

Plant growth regulators mg L^{-1}			Regeneration culture %	No of shoot/explant (Mean ±SE)
BA	KIN	NAA		
0.0	0.0	0.0	61	1.0±0.0^{h}
0.1	0.0	0.0	94	2.0±0.2^{b}
0.5	0.0	0.0	100	2.0±0.2^{b}
1.0	0.0	0.0	100	2.7±0.1^{a}
1.5	0.0	0.0	72	1.9±0.2^{b}
3.0	0.0	0.0	56	1.4±0.2^{de}
4.0	0.0	0.0	50	1.3±0.2^{ef}
0.0	0.1	0.0	85	1.0±0.0^{h}
0.0	0.5	0.0	89	1.1±0.0^{gh}
0.0	1.0	0.0	89	1.2 ±0.0^{fg}
0.0	1.5	0.0	90	1.4 ±0.1^{de}
0.0	3.0	0.0	94	1.5 ±0.1^{d}
0.0	4.0	0.0	94	1.7±0.1^{c}
0.1	0.0	0.1	28	0.3±0.1^{ef}
0.5	0.0	0.1	33	0.4±0.2^{i}
1.0	0.0	0.1	33	0.4±0.2^{i}
1.5	0.0	0.1	55	0.7±0.2^{jk}
3.0	0.0	0.1	55	0.7±0.2^{jk}
4.0	0.0	0.1	61	1.1±0.2^{gh}
0.1	0.0	0.5	28	0.4±0.2^{i}
0.5	0.0	0.5	22	0.2±0.1^{m}
1.0	0.0	0.5	22	0.2±0.1^{m}
1.5	0.0	0.5	11	0.1±0.1^{n}
3.0	0.0	0.5	callus	0.0±0.0^{n}
4.0	0.0	0.5	callus	0.0±0.0^{n}
0.1	0.0	1.0	callus	0.0±0.0^{n}
0.5	0.0	1.0	callus	0.0±0.0^{n}
0.0	0.1	0.1	56	0.6±0.1^{k}
0.0	0.5	0.1	67	0.7±0.1^{jk}
0.0	1.0	0.1	44	0.4±0.1^{l}
0.0	1.5	0.1	39	0.4±0.1^{l}
0.0	3.0	0.1	callus	0.0±0.0^{n}
0.0	4.0	0.1	callus	0.0±0.0^{n}
0.0	0.1	0.5	72	0.7±0.1^{jk}
0.0	0.5	0.5	60	0.3±0.1^{lm}
0.0	1.0	0.5	58	0.3±0.1^{lm}
0.0	1.5	0.5	56	0.6±0.1^{k}
0.0	3.0	0.5	callus	0.0±0.0^{n}
0.0	4.0	0.5	callus	0.0±0.0^{n}

Table 3: Effect of different sesame Genotypes on shoot induction cultured on regeneration media after six weeks.

Cultivar	Regeneration culture %	No of shoot/explant (Mean ±SE)
Bromo Ky	100	2.7±0.1^{a}
Elgadareif 1	100	1.0±0.0 ^{c}
Elabassia karma	88	1.1±0.1^{c}
Asowad	83	0.9±0.1^{d}
Abusitta	88	0.9±0.1^{d}
Elobaied	100	1.4±0.1^{b}
Khidir	94	1.0±0.1^{c}
Abusandog	83	0.8±0.1^{e}
Ali Mahdi	83	0.9±0.1^{d}
Beladi	72	0.8±0.1^{e}

Result for shoot induction capacity of 10 sesame cultivars in the regeneration media is presented in table3. There were significant cultivar differences in the percentage of shoot regeneration and the number of shoots produced per explant. The best cultivar Promo ky scored a frequency of 100% and produced on average 2.7 shoots per explant. The lowest regeneration frequency (72%) and number of shoots produced per explant (0.8) were recorded for Beladi cultivar. Cultivar differences have also been reported in past studies on sesame (Rao and Vaidyanath, 1998a, b; Gangopadhyay et al.,1998). This may suggest that there is a considerable variation in the distribution of the genes responsible for multiple shoot formation among different populations of sesame. For *In vitro* rooting of the regenerated shoots, NAA was found to be the most effective auxins for root induction compared to basal media without or with IAA or IBA. Among the different concentrations of NAA, 0.05 mg L^{-1} gave the highest rooting percentage (100%) and the highest no of root /shoots (63.1) (Table 4). The promotive effect of reducing the salt concentration of basal medium and using of NAA on rooting of *in vitro* induced shoots was already reported by (Baskaran and Jayabalan,2003; Baskaran and Jayabalan ,2006)

After well established root (Fig.1B) plantlets were subjected acclimatization. Plant removed from rooting medium after six weeks of incubation and transferred to plastic pots containing autoclaved soil (soil:sand 2:1) and covered with glass bottle (Fig.1C) to maintain humidity then kept under culture room conditions for one week. After three weeks, glass bottles were removed and transferred to green house and placed under shade until growth was observed. 90% of the plants survived and all were morphologically normal (Fig.1D).

Table 4: Effect of auxins on rooting of *in vitro* derived shoots of *Sesamum indicum* , cv promo ky after six week of culture.

Auxin (mg/L)			Rooting %	No of root/shoot (mean ±SE)
NAA	IAA	IBA		
0.0	0.0	0.0	100	4.5±0.4 ^{d}
0.025	0.0	0.0	100	45±2.3 ^{b}
0.05	0.0	0.0	100	63±3.2 ^{a}
0.5	0.0	0.0	callus	0.0±0.0 ^{f}
1.0	0.0	0.0	callus	0.0±0.0 ^{f}
0.0	0.025	0.0	100	6.8±0.3 ^{c}
0.0	0.05	0.0	88	7.8±0.3 ^{c}
0.0	0.5	0.0	88	8.4±0.3 c
0.0	1.0	0.0	callus	0.0±0.0 ^{f}
0.0	0.0	0.025	75	2.5±0.2 ^{e}
0.0	0.0	0.05	83	3.6±0.2 ^{cd}
0.0	0.0	0.5	callus	0.0±0.0 ^{f}
0.0	0.0	1.0	callus	0.0±0.0 ^{f}

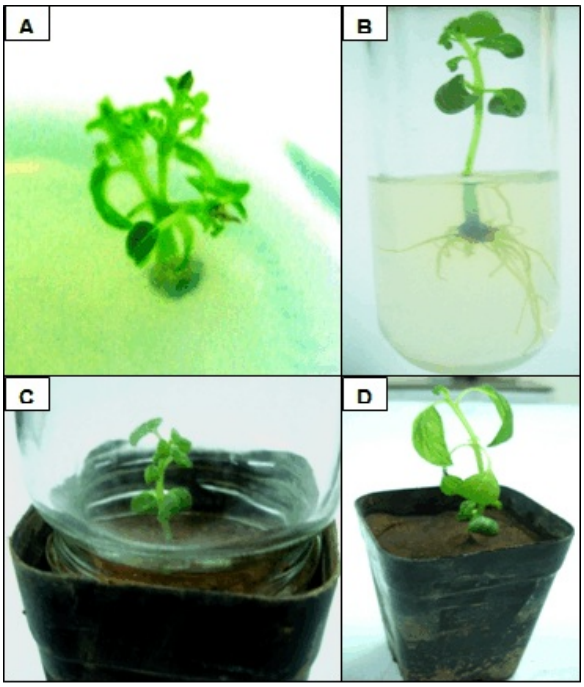

Fig. 1: *In vitro* induction of multiple shoots and plant regeneration of sesame (*Sesamum indicum*) cultivar (Promo Ky),
A- Multiple shoots induced from shoot tip explant
B- *In vitro* rooted shoot on half – strength MS basal medium supplemented with NAA (0.05 mg L^{-1}),
C- Potting up and hardening of the plantlets
D- Sesame plant established in soil.

Conclusions

The findings of the present study are of considerable significance, since it has described a regeneration system for a Sudanese sesame cultivar which has not previously been reported. Moreover, the usage of BA as cytokinin for *in vitro* multiplication and NAA as the auxin for induction of roots is very beneficial due to the fact that BA and NAA are very effective and less expensive plant growth regulators and can safely be autoclaved (Thomas and Blaksley 1987). Therefore, the results obtained here could be useful in improving this economically valuable crop.

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References

Abdellatef, E. and M.M. Khalafallah, 2007. Adventitious shoot formation and plant. regeneration in medium staple cotton (*Gossypium hirsitum* L.) cultivar (Barac B-67). Int. J. Agri. Biol., 9(6): 913- 916.

Ashri, A., 1998. Sesame breeding. Plant Breeding Reviews., 16:179-228.

Baskaran, P. and N. Jayabalan, 2003. Effect of growth regulators on multiple shoot production in *Sesamum indicum L*. Indian journal of plant physiology., (special issue): 639-642.

Baskaran, P. and N. Jayabalan, 2005. An efficient plant regeneration system for Sorghum bicolor a valuable major cereal crop. Plant Biotechnology., 7:247-257.

Baskaran, P. and N. Jayabalan, 2006. *In vitro* mass propagation and diverse callus orientation on *(Sesamum indicum* L.) an important oil plant. Journal of Agricultural Technology., 2(2):259-269.

Bedigian, D. and J. Harlan, 1986. Evidence for cultivation on sesame in the ancient world. Economic Botany., 40:137-154.

Bhojwani, S.S., 1980. Micropropagtion method for a hybrid willow (*Salix matsudana x alba* NZ-1002). New Zealand Journal of Botany., 18: 209-214.

Brar, G. and R. Ahuja, 1979. Sesame: its culture, genetics, breeding and biochemistry. Annu. Rev. Plant. Sci., 285-313.

Devi, Y.S., B.B. Mukherjee and S. Gupta, 1994. Rapid cloning of elite teak (*Tectona grandis* Linn.) by *in vitro* multiple shoot production. Indian Journal of Experimental Biology., 32: 668-671.

Droste, A., S.A. Machado, V.A. Matos and W.J. Almeida, 2005. *In Vitro* Culture of *Vriesea gigantean* and *Vriesea Philippocoburgii*: Two Vulnerable Bromeliads Native to Southern Brazil. Brazilian Arch. Biol. Technol., 48: 717-22.

Duncan, D.B., 1955. Multiple range and multiple F test. Biometrics., 11: 1-42.

FAO: FAO stat Databases 2005 [http://faostat.fao.org/].

Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultres of soybean root cell. Exp. Cell Res., 50: 151-8.

Gangoadhyay, G., R. Poddar, S. Gupta, 1998. Micropropagation of sesame (*Sesamum indicum L*.) by *in vitro* multiple shoot production from nodal explants. Phytomorphology., 48: 83-90.

George, L., V.A. Bapat and P.S. Rao, 1987. *In vitro* multiplication of sesame (*Sesamum indicum*) through tissue culture. Ann. Bot., 60: 17-21.

George, L., V.A. Bapat, and P.S. Rao, 1987. *In vitro* multiplication of sesame *(Sesamum indicum* L.*)* through Tissue culture. Annals of Botany., 60: 17-21.

George, L., V.A. Bapat, and P.S. Rao, 1989. Plant regeneration *in vitro* in different cultivars of sesame (*Sesamum indicum* L.). Proceeding of Indian Academy Science., 99: 135-137.

Gulati, A. and P.K. Jaiwal, 1992. *In vitro* induction of multiple shoots and plant regeneration from shoots tip of mung bean (*Vigna radiate* (L.) Wilczek). Pl. cell tiss. Org. Cult., 29: 199-205.

Khalaflla, M.M. and K. Hattori, 2000. Differential *in vitro* direct shoot regeneration responses in embryo axis and shoot tip explant of faba bean. Breed. Sci., 50: 117-22.

Laurentin, H.E. and P. Karlovsky, 2006. Genetic relationship and diversity in a sesame (*Sesamum indicum L*.) germplasm collection using amplified fragment length polymorphism (AFLP). BMC Genetics., 7: 10.

Murashige, T. and F. Skoog, 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. Physiol. Plnt., 15: 473-9.

Rao, K.R. and K. Vidyanath, 1997. Induction of multiple shoots from seedling shoot tips of different varieties of Sesamum. Indian J. Plant physiol., 2: 257-261.

Rao, K.R. and K. Vaidyanath, 1998a. Somatic embryogenesis and regeneration from different explants of *Sesamum indicum* L .National Symp. Commercial aspects of plant tissue culture, Molecular Biology and Medicinal Plant Biotechnology, Jamia Hamdard, New Delhi.

Rao, K.R. and K. Vaidyanath, 1998b. Synseed and micropropagation in Sesamum. National Symposium on "Perspectives in biotechnology" held at the Department of botany, Kakatiya University Warangal, India.

Sakhanokho, H.F, A. Zipf, K. Rajasekaran, S. Saha and G.C. Sharma, 2001. Induction of highly embryogenic calluses and plant regenertion in Upland and Pima cottons. Crop Sci., 41: 1235-1240.

Sharma, M. and L.K. Pareek, 1998. Direct shoot bud differentiation from different explants of *in vitro* regenerated shoots in sesame. J. Phytol. Res., 11: 161-163.

Taskin, K.M. and K. Turgut, 1997. *In vitro* regeneration of sesame (*Sesamum indicum* L.). Tr. J. Bot., 21: 15-18.

Thiem, B., 2003. *In vitro* propagation of isoflavone-producing *Pueraria lobata* (Willd.) Ohwi. Plant Science., 165: 1123-1128.

Thomas, T.H. and D. Blakesley, 1987. Practical and potential uses of cytokinins in agriculture and horticulture. Brit. Plant Growth Regul. Group Mono., 14: 69-83.

Vengadesan, G., A. Ganapathi, R.P. Anand, N. Selvaraj, 2003. *In vitro* propagation of *Acacia sinuate* (Lour.) Merr. from nodal segments of a 10-year-old tree. In Vitro Cellular and Developmental Biology - Plant., 39: 409-414.

Welander, M., N.T. Welander and A.s. Brackman, 1989. Regulation of *in vitro* shoot multiplication in syringe, Alnus and Malus by different carbon sources, Horticulture Science., 64: 361-366.

Were, B.A.S. Gudu1, A.O. Onkware, A.S. Carlsson and M. Welander, 2006. *In vitro* regeneration of sesame (*Sesamum indicum* L.) from seedling cotyledon and hypocotyl explants. Plant Cell, Tissue, and Organ Culture., 85: 235-239.

Younghee, K., 2001. Effects of BA, NAA, 2,4-D and $AgNO_3$ treatments on the callus induction and shoot regeneration from hypocotyl and cotyledon of sesame (*Sesamum indicum* L.). J. Korean Soc. Hort. Sci., 42: 70-74.