Pure Appl. Biol., 9(1): 303-309, March, 2020
http://dx.doi.org/10.19045/bspab.2020.90035

Research Article

In vitro shoots multiplication from nodal explants of Sesame (*Sesamum indicum* L.)

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Citation

Muhammad Asad, Nadeem Ahmed, Amir Sohail, Tanveer Burni, Fazal Hadi, Rehman Ali, Muhammad Nauman Khan, Abdullah Aziz and Abbas Muhammad. *In vitro* shoots multiplication from nodal explants of (*Sesamum indicum* L.). Pure and Applied Biology. Vol. 9, Issue 1, pp303-308. http://dx.doi.org/10.19045/bspab.2020.90035

Received: 12/01/2019 Revised: 06/09/2019 Accepted: 23/10/2019 Online First: 06/11/2019

Abstract

An effective micro propagation system has been developed efficiently through an enriched culture system for *Sesamum indicum* L. used as a medicinal plant and spicy crop significant from a nutritional point of view. The multiplication of shoot was obtained on Murashige and Skoog medium supplemented with 15 g l-1 sucrose, 8 g l-1 agar and fortified with benzyl amino purine (BAP) in rabies of 0.1 -1 mg / l. The maximum number of young shoots was appeared on MS medium supplemented with 0.5 mg / l benzyl amino purine (BAP). Multiple combinations of harmonics were used for multiple inductions. Developed micro-shoots were shifted to culture medium supplemented with IAA (1.0 mg / l) for efficient rooting. For hardening newly developed shoots were carried out and 71% were survived effectively. This system can be successfully applied for mass propagation of *Sesamum indicum*.

Keywords: BAP; Medium MS; Micro propagation; Multiple shoots; Sesame

Introduction

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae and known as the oldest crop of oilseeds. Seasame cultivated in Afghanistan India, Pakistan, Russia, China, South America and some African countries **[1]**. Through worldwide sesame cultivated on a total area of over 7.7 million hectares with entire production of 3.3 million tons **[2]**. Seeds of sesame are vital source of oil and used as spices. It consist of 48-58% of oil, it is very stable due to naturally occurring antioxidants sesamines, sesamol and sesamolin **[3]**. Sesame oil is used in the Ayurvedic medicine system. Sesame seeds have antioxidant and antitumor potential. Due to abundant amount of protein the oil cake is used for livestock feed **[4]**.

Sesamum indicum is highly uncooperative to regenerate in vitro conditions. Though, several procedures for micro propagation **[5, 6]**, somatic embryos **[7]**, and indirect regeneration of adventitious **[8, 9]**, were achieved with low frequency. The productivity of *Sesamum indicum* L. is relatively low compared to other oleaginous crops because the cultivation of sesame is limited by poor soils **[10, 11]**. The present

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study shows the results of an experiment to develop an appropriate procedure for the regeneration of multiple shoots in *Sesamum indicum* L.

***In vitro* mass propagation**

Plan tissue culture plays an important role in the study of a growing number of basic and practical programs in plant sciences [12]. Globally tissue culture techniques are used for plant conservation [13]. In this study we propose the conservation and rapid multiplication of Sesame for its use.

Materials and methods

Sesame (*Sesamum indicum* L.) seeds for experimental work have been obtained from the University of Agriculture of Peshawar. Seeds were germinated on Murashige and Skoog (MS) media. Media were pH adjusted before autoclaving at 121°C, 1 atm. The cotton bed method was used with water and MS media for the in vitro regeneration of seedlings from seeds. All materials used in cultural work must be free of microbes. This is achieved by one of the following approaches: flame sterilization, 70% ethanol cleaning and other surface sterilants. The tip of shoot and the nodal region of in vitro derived seedlings were collected for the induction of multiple shootings. The surface-sterilized seeds were transferred to Murashige and Skoog (MS) medium integrated with 0.8% agar, 3% sucrose and 1% of myinositol. The media pH was adjusted to 5.7 and the maximum growth of the seedlings was observed and was subsequently sub cultured into the medium containing variable concentrations of BAP, IAA and NAA. The subculture process was carried out with the tip of the shoot and the nodal part of the plants. Therefore, It has been optimized with the maximum shoot lets formed from the sub cultured excised shoot tips. The maximum shooting length and internodes have been determined.

Results and discussion

The seeds that showed in vitro regeneration responded successfully. On the 2nd day the seeds were germinated and from the 7th day the maximum growth rate was reached. Plants grown in vitro have been used for in vitro cultivation studies [3]. The high frequency of germination was recorded on MS basal media similar results were obtained in the *in vitro* regeneration of Sesame by [14].

In *Sesamum indicum* L. both apical and all nodal segments were used as primary explants, the nodal explant is the most commonly used as explant to produce micro shoot [15, 16]. In recent study shoots multiplication from nodal explants was achieved on media supplemented with 0.5 mg / l benzyl amino purine (BAP) and 1.5 mg / l of KIN show high frequency. (84%) Of induction of multiple shoots (Table 3, Fig. 2). 88% Shoots multiplication was achieved by supplement media of 0.5mg/1 BAP and 1.5mg/1 NAA (Table 2). The same percentage (88%) was also recorded in 0.5mg/1 BAP and 1.5mg/1 IBA media (Table 4).These results were in agreement with [5, 9, 17].

The well-rooted seedlings were transferred to the potting mix in the ratio of sand: soil: farm manure in a 1: 1 ratio: 1. 70% (Table 1) of the germination percentage was recorded.

In a recent experiment, an effective and reproducible procedure for the multiplication standardization of (*Sesamum indicum* L. CVV SVPR-1) was cultivated and formulated a comparatively efficient and reproducible shoot induction system has been worked out utilizing nodal and apical bud explants.Root induction was performed to increase the efficiency of sesame.

The study revealed that apical shoots and nodal segments for explantation were achieved in *Sesamum indicum* L. On Murashige and Skoog medium integrated with benzylaminopurin (BAP) (Table 5, Fig. 1).(0.5 mg / l) more, Indole acetic acid (IAA)

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(1.0 mg / l) *.in vitro* cultured micro shoots were successfully developed and transferred to the potting mix in the sand ratio: soil: courtyard manure (Fig. 3).

Table 1. Effect of BAP on multiplication of shoots per shoot tip cultured on MS media

Harmone BAP(mg/l)	Total No. of explants	No. of explants responded	Percentage of responding cultures	M±SD
0.1	50	24	48%	2.16±1.158
0.2	50	35	70%	2.40±1.130
0.3	50	41	82%	3.31±2.198
0.4	50	38	76%	2.26±1.114
0.5	50	48	96%	4.48±3.292
0.6	50	34	68%	2.98±2.341
0.7	50	29	58%	1.46±1.198
0.8	50	34	68%	2.83±2.106
0.9	50	40	80%	3.09±2.347
1.0	50	32	64%	2.67±2.159

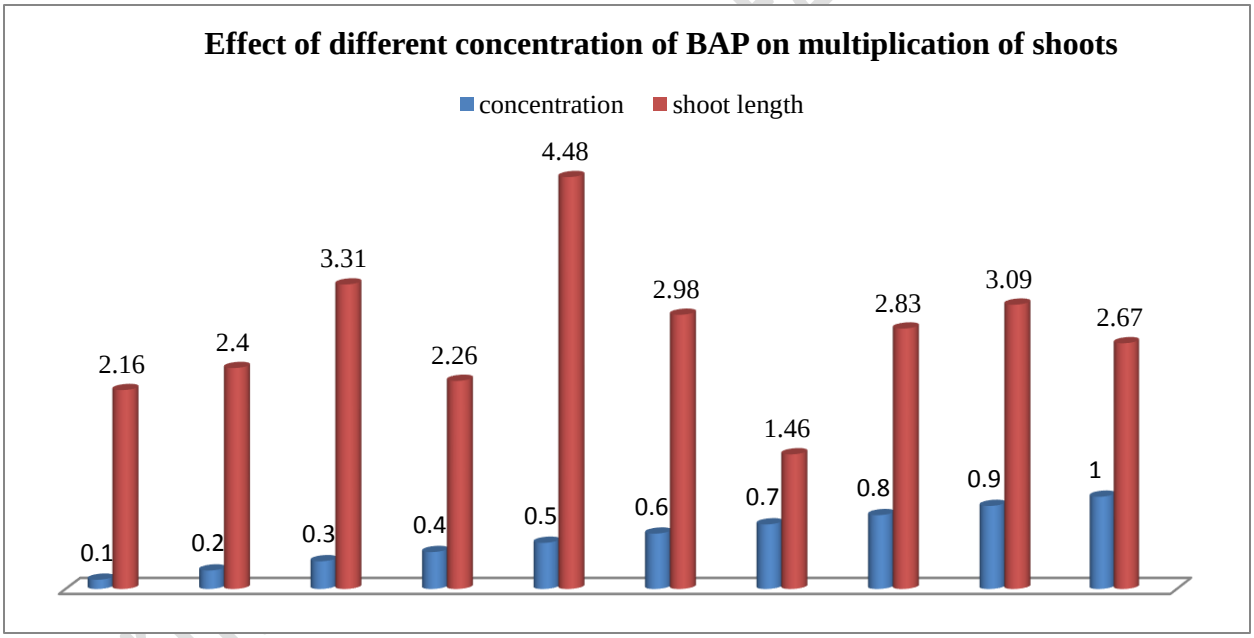

Figure 1. Effect of different concentration of BAP on multiplication of shoots

Table 2. Effect of BAP and NAA on multiplication of shoots per shoot tip as explants

Conc. of BAP (mg/l)	Conc. of NAA (mg/l)	Total No. of explants	Percentage of responding cultures	Percentage of responding cultures	M±SD
0.5	0.5	50	32	64%	3.27±2.742
0.5	1.0	50	38	76%	3.16±2.483
0.5	1.5	50	44	88%	4.45±3.946
0.5	2.0	50	23	46%	2.54±2.06
0.5	2.5	50	18	36%	1.78±1.53

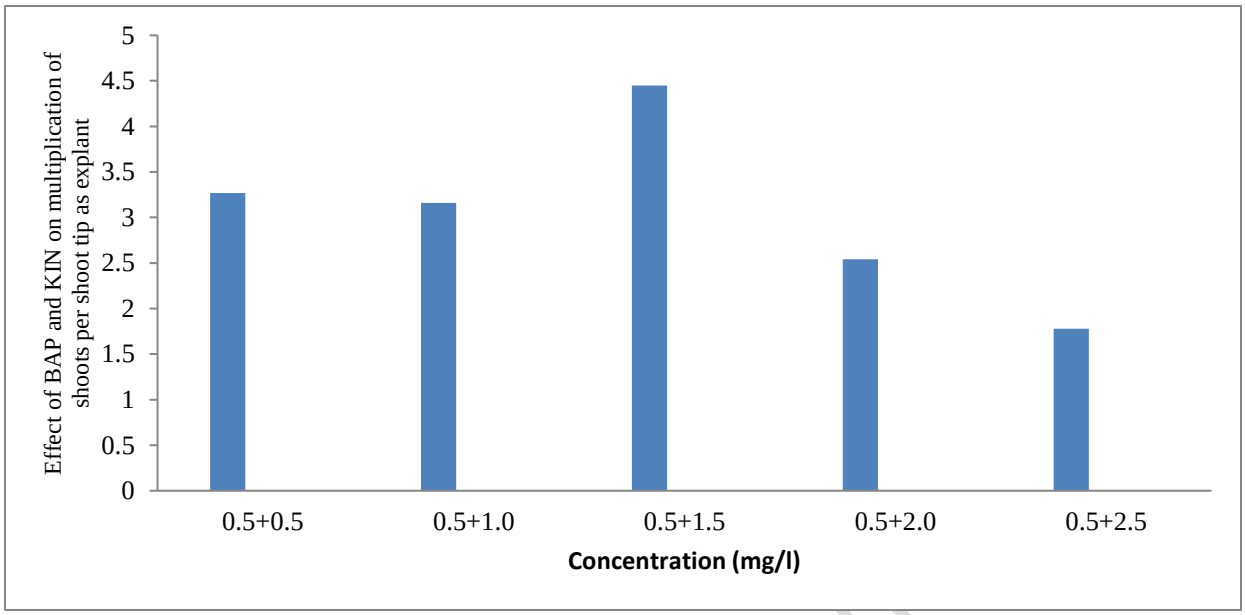

Figure 2. Effect of BAP and KIN on multiplication of shoots per shoot tip as explant

Table 3. Effect of BAP and KIN on multiplication of shoots per shoot tip as explants

BAP (mg/l)	KIN (mg/l)	Total No. of explants	No. of explants responded	Percentage of cultures responding	M±SD
0.5	0.5	50	37	74%	3.48±2.651
0.5	1.0	50	24	48%	3.14±2.63
0.5	1.5	50	42	84%	4.66±3.254
0.5	2.0	50	20	40%	2.87±2.16
0.5	2.5	50	15	30%	2.38±1.59

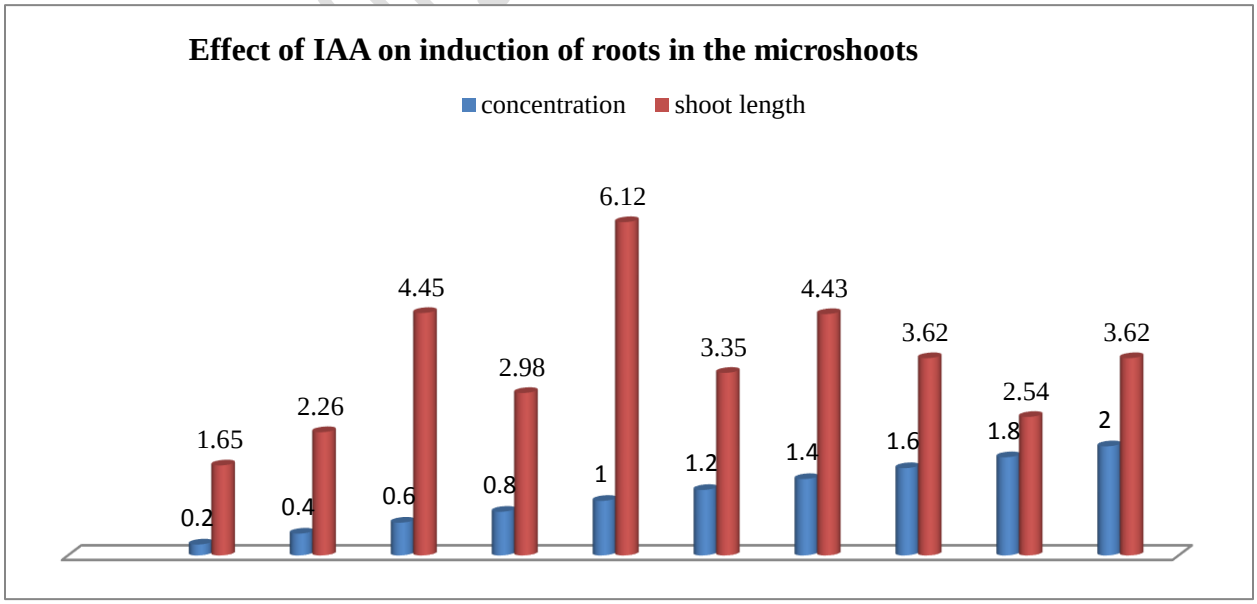

Figure 3. Effect of IAA on induction of roots in the micro shoots

Table 4. Effect of BAP and IBA on multiplication of shoot per shoot tip as explants

BAP (mg/l)	IBA (mg/l)	Total No. of explants	No. of explants responded	Percentage of responding cultures	M±SD
0.5	0.5	50	25	50%	3.83±2.492
0.5	1.0	50	18	36%	3.36±2.571
0.5	1.5	50	44	88%	4.57±3.635
0.5	2.0	50	27	54%	3.68±2.051
0.5	2.5	50	37	74%	3.24±2.856

Table 5. Effect of IAA on root induction per shoot tip cultured on MS medium

Hormone IAA(mg/l)	Total No. of explants	No. of explants responded	Percentage of responding cultures	M±SD
0.2	50	18	36%	1.65±0.986
0.4	50	23	46%	2.26±2.187
0.6	50	36	72%	4.45±3.685
0.8	50	22	44%	2.98±2.602
1.0	50	45	90%	6.12±5.062
1.2	50	21	42%	3.35±2.517
1.4	50	29	58%	4.43±3.253
1.6	50	18	36%	3.62±2.163
1.8	50	14	28%	2.54±1.967
2.0	50	27	54%	3.62±2.385

Conclusion

Our Present performance determine the culture conditions for micro propagation of sesame plant and concluded that shoot regeneration of *Sesamum indicum* L. were achieved in *vitro* on MS medium which containing different concentrations of cytokinins and auxins. It is also suggested that high frequency of shoot growth will be achieved by means of BAP, IBA and IAA application. This work will be also provide a helpful tool for genetic transformation and reproductive growth and for others tissue culture studies.

Authors' contributions

Conceived and designed the experiments: N Ahmed, Performed the experiments: M Asad, Analyzed the data: T Burni, F Hadi, R Ali & MN Khan, Contributed materials/ analysis/ tools: A Aziz & A Muhammad, Wrote the paper: A Sohail.

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