In Vitro Mass Propagation of Salvia (*Salvia splendens*) from Nodal Explant

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Abstract

A high frequency *in vitro* plant regeneration was established in salvia on MS medium supplemented with 1.0 mg/l BA + 0.5 mg/l Kin + 0.2 mg/l GA_3 using nodal segment as explants. Ninety percent of the explants responded to form shoots culture after 90 days. The average number of shoot per explant was 8.0 ± 0.40 and the average shoot length of 6.50 ± 0.70 cm were observed in this medium. Shoots rooted well when they were excised individually and implanted on half strength of MS medium supplemented with 0.5 mg/l IBA, in which 90% shoot induced roots. The average number of root per shoot was 12.0 ± 0.90 and the average root length of 7.5 ± 1.50 cm were observed in this medium culture after 30 days.

Keywords: Micropropagation, node culture, salvia

1. Introduction

Salvia splendens is an ornamental plant, native to Brazil and belongs to the family labiateae [1]. It is commonly known as red salvia or red sage or scarlet salvia and grown annually in the winter [2]. Ornamental bedding plants include annual and perennial plant species may provide seasonal colours to landscapes and homegardens [3]. Among a wide variety of bedding plants, *Salvia splendens* Ker-Gawl is most commonly used mainly due to their brilliant colour during the winter season [4]. The flowers of *Salvia splendens* grown on spikes, which are quite showy with attractive looking and thus its popularity. This species is using into the landscape, container gardens and striking border as bedding plants [5]. *Salvia splendens* is quite limited in their colour type to white, salmon, purple and the traditional fresh red. The plant also relatively sensitive to high temperature [4]. It is obvious that development of new *Salvia splendens* variety with other colours and heat tolerance is imperative for increasing their ornamental and economic value. Besides, other species of the genus salvia is widely used in medicine, cosmetology and food industry [6-8]. In Bangladesh, the species *Salvia splendens* is commonly available and mostly found to landscaping in the home garden, schools, colleges, universities, officies, industries, parks and along the high ways to beautify the environment with improve environmental protection and also to promote living quality and healthy body. It is mainly propagated by seeds or stem cutting. This traditional method of propagation is limited due to seasonal barrier and climatic factor and also the propagules carry insect-pest-diseases. Some other species of the genus salvia have also been reported to propagated *in vitro* method [9-14]. To our knowledge, very little work was done concerning the micropropagation of this species. In this context, an attempt was made to develop an efficient *in vitro* propagation protocol for *Salvia splendens* in contributing to the commercial cultivation and conservation and also to create genetic variants using *in vitro* mutagenesis and genetic engineering.

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2. Materials and Methods

One month old young nodal explants of *Salvia splendens* was collected from the Plant Biotechnology & Genetic Engineering Division experimental field at Atomic Energy Research Establishment, Savar, Dhaka. The explants were then washed thoroughly with the detergent 'Trix' and kept for 20 minutes under running tap water to eliminate dirt and organisms. The nodal explants were surface sterilized by treating with an aqueous solution of 0.1% mercuric chloride accompanied with 2 drops of 'tween 20' for 10 minutes in the laminar air flow cabinet under aseptic conditions. Rinsing was done 4 times with sterile distilled water. Sterilized nodal segments were then cultured on MS media with different concentrations of BA and 2iP alone and also different concentrations and combinations of BA + IAA, BA + Kin and BA + Kin + GA_3 for multiple shoot induction. Subculture was done 30 days interval on the same medium for promoting strong and healthy multiple shoots. Morphologically strong and healthy proliferated shoots were excised individually and transferred to half strength of MS basal media supplemented with different concentrations of IBA, IAA and NAA for root induction. The sucrose (table sugar) concentration was used 30 g/l and the pH of the media adjusted to 5.8 prior to autoclaving. Cultures were incubated at 26 ± 2ºC with a 16 hour illumination of 21.8 µmol/cm^2/s provided by cool white fluorescent tubes. The experiment was conducted with 10 explants per media type. Data were collected on different characters at day 90 for multiple shooting and at day 30 for rooting of shoots. Observations on culture were carried out every alternate day.

3. Results and Discussion

Shoot initiation from the nodal explants (Fig. 1) was observed in most of the media used in the study. It was found that shoot proliferation and multiplication differed according to media component used (Table 1). Among the media component used, the combination of 1.0 mg/l BA and 0.5 mg/l Kin + 0.2 mg/l GA_3 was found to be the best for shoot proliferation and multiplication (Fig. 2), in which 90% explants produced shoots culture after 90 days. It is

obvious that this concentration is optimum for normal shoot growth and development of *Salvia splendens*. The average number of shoot per explant was 8.0 ± 0.40 and the average shoot length of 6.50 ± 0.70 cm were achieved in this medium culture after 90 days. These results are similar to the findings in *Salvia officinalis* [6]. Combination of BA + IAA resulted in the highest shoot regeneration in other species of salvia was also reported [15]. The media supplemented with BA were the most effective in promoting shoot development in other species of salvia were observed [11, 16]. Cytokinin-auxin combination has been widely used for plant regeneration of other members of the labiatae family [17). These are in agreement with the present study. Reported to observed plantlets from cotyledon derived callus in *Salvia splendens* [18]. This indicates that *in vitro* mass propagation and genetic improvement is imperative of this plant. Rooting response differed according to concentration of different auxins used (Table 2). Among the auxins used, IBA was found most responsive and 0.5 mg/l was observed optimum, in which 90% shoots rooted (Fig. 3) within 30 days of culture in this medium. The average number of root per shoot was 12.0 ± 0.90 and the average root length of 7.5 ± 1.50 cm were recorded in this medium culture after 30 days. Good response towards root induction of *Salvia splendens* and other species of salvia by using IBA was also reported [18, 19, 16]. *In vitro* raised shoots responded well for root induction at low concentrations of IBA and did not response at all when the concentrations increased to 2.50 mg/l and higher than that. This indicates that high concentrations of IBA is toxic for rooting tissue of *Salvia splendens*. The superiority of IBA for rooting over other auxins has also been reported [20-23]. Comparatively healthy rooted shoots were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *in vitro* rooted plantlets were then transferred to poly bags (Fig. 4) containing a mixture of soil and compost (2:1) and covered with transparent polyethylene lid to maintain high humidity. After one week, the polyethylene lids were removed and plantlets were kept in a shade and misted twice a day. About 80% of the plantlets were resumed new growth within 30 days. A total number of 40 plantlets were survived in the field out of 50 *in vitro* regenerants. The *in vitro* raised plantlets was observed to flowering (Fig. 5) at 120 days after transferring in the field.

Figs. 1-5. *In vitro* plant regeneration of *Salvia splendens* through the culture of nodal segments, (1) Shoot initiation on MS + 1.0 mg/l BA and 0.5 mg/l Kin + 0.2 mg/l GA_3 culture after 30 days, (2) Strong and healthy multiple shoot formation on transferring to the same medium containing MS + 1.0 mg/l BA and 0.5 mg/l Kin + 0.2 mg/l GA_3 culture after 90 days, (3) Root induction on transferring to half strength of MS + 0.5 mg/l IBA culture after 30 days, (4) *In vitro* raised plant in poly bag after 30 days of acclimation period and (5) *In vitro* raised plant at flowering state acclimation after 120 days.

Table 1. Effect of different concentrations and combinations of plant growth regulators on *in vitro* shoot induction of salvia using nodal segment as explants at 90 days

Different concentrations and combinations of plant growth regulators in MS media (mg/l)	% of explants induced shoots	Average number of shoot induced/explant Mean ± SE	Average shoot length (cm) Mean ± SE
BA			
1.0	20	2.0 ± 0.10	2.0 ± 0.10
2.0	20	2.0 ± 0.30	2.5 ± 0.10
3.0	-	-	-
4.0	-	-	-
2iP			
1.0	10	2.0 ± 0.20	2.0 ± 0.20
2.0	20	2.0 ± 0.70	2.5 ± 0.40
3.0	20	2.5 ± 0.60	2.0 ± 0.20
4.0	-	-	-
BA + IAA			
0.5 + 0.1	40	2.0 ± 0.40	2.0 ±0.10
1.0 + 0.1	70	5.0 ± 0.60	5.0 ± 0.60
1.5 + 0.1	50	3.0 ± 0.40	2.75 ± 0.45
2.0 + 0.1	20	2.0 ± 0.20	2.5 ± 0.60
BA + Kin			
0.5 + 0.5	60	6.0 ± 0.40	4.5 ± 0.60
1.0 + 0.5	50	6.0 ± 0.70	3.5 ± 0.20
1.5 + 0.5	20	2.0 ± 0.30	2.5 ± 0.50
2.0 + 0.5	-	-	-
BA + Kin + GA_3			
0.5 + 0.5 + 0.2	60	4.0 ± 0.30	3.5 ± 0.40
1.0 + 0.5 + 0.2	90	8.0 ± 0.40	6.5 ± 0.70
1.5 + 0.5 + 0.2	40	2.0 ± 0.20	2.5 ± 0.60
2.0 + 0.5 + 0.2	20	2.0 ± 0.30	2.0 ± 0.30

Table 2. Effect of IBA, IAA and NAA on half strength of MS media in root induction of *in vitro* raised shoots of salvia at 30 days

Name of hormones	Concentrations (mg/l)	% of shoot induced roots Mean ± SE	Average number of root induced/shoot Mean ± SE	Average root length (cm) Mean ± SE
IBA				
	0.5	90	12.0 ± 0.90	7.5 ± 1.5
	1.0	60	8.0 ± 0.60	6.5 ± 0.60
	1.5	30	5.0 ± 0.70	5.0 ± 0.30
	2.0	10	3.0 ± 0.10	4.5 ± 0.40
IAA				
	0.5	10	3 ± 0.20	4.0 ± 0.20
	1.0	10	3 ± 0.10	2.0 ± 0.10
	1.5	-	-	-
	2.0	-	-	-
NAA				
	0.5	-	-	-
	1.0	10	3.0 ± 0.20	2.5 ± 0.50
	1.5	-	-	-
	2.0	-	-	-

4. Conclusion

Salvia splendens is an attractive and colourful bedding ornamental plant due to its showy looking. The protocol described in this study is reproducible and providing disease free propagules of an elite clone for commercial cultivation whilst adequate planting material of an elite clone is concern in the country. From the above investigation it may be concluded that among the growth regulators used in the study 1.0 mg/l BA + 0.5 mg/l Kin + 0.2 mg/l GA_3 was found most effective for multiple shoot induction and half strength MS + 0.5 mg/l IBA appeared better results for root induction from shoots. Therefore, this protocol would be facilitate year round mass propagation, long-term *in vitro* regeneration, conservation, international germplasm exchange, creation of somaclonal variants and genetic improvement of this brilliant colourful ornamental plant through *in vitro* mutagenesis and genetic transformation.

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