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In vitro mass propagation of *Withania somnifera* Dunal using seaweed extract

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ABSTRACT

The present study was aimed to develop a cost-effective and efficient protocol for mass propagation of high-quality seedlings through tissue culture by using seaweed extract as biostimulants instead of synthetic chemicals. The nodal explant of field grown *W. somnifera* estabilised on Murashige and Skoogs medium (MS) and Gamborg B5 medium supplemented with six concentration of 2,4 D. The percentage of culture response from the nodal explant ranged from 44 to 80 and 3.0 mg l^{-1} 2,4 D found to be best for callus induction. MS media containing different concentration of seaweed extract (10, 20, 40, 60, 80 and 100 %) were tested individually for shoot induction. The medium supplemented with 40 % seaweed extract exhibited maximum number of shoots with about 8.6 shoots/ callus and 80 % seaweed extract exhibited 4.3 shoots/ callus. It is evident from this study that seaweed extracts can be used as substitute for synthetic growth hormones for micropropagation of medicinally important plant *W. somnifera* for clonal propagation and conservation.

***Keywords*:** Seaweed; Propagation; M.S.Medium-B5 medium; *Withania somnifera*

1. INTRODUCTION

Withania somnifera (L.) Dunal. of Solanaceae, is a valuable herb used in traditional Ayurvedic medicine and is often taken for its nervous sedative, hypnotic, tonic, astringent and aphrodisiac properties (Matsuda, 2000; Winters, 2006). Ashwagandha roots are a constituent of over 200 formulations in Ayurveda, Siddha and Unani medicine, which are used in the treatment of various physiological disorders.This drug is known to have anti-inflammatory, antitumor, antioxidant, anticonvulsive, and immunosuppressive properties (Baldi *et al*., 2008). Multiple uses of the plant have necessitated its large-scale collection as raw material to the medicine industry, leading to progressively declining and making it anendangered plant species. Commonly *W. somnifera* propagated commercially by the means of seeds because of the lack of natural ability for vegetative propagation (Sen and Sharma, 1991). Percentage of seed germination decreases drastically with time. A protocol developed for micropropagation through tissue culture could possibly be used for clonal propagation and conservation.

In vitro plant regeneration has been found to depend on many factors, *viz*. genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and

quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed, 1999). Of which the chemicals used in the preparation of basic medium as a nutrient source and the growth regulators are costly and determines the frequency of plant regeneration, depending on the type of explants and concentration of growth regulators added to the regeneration medium. Besides commercially available auxins and cytokinins for in vitro propagation, an alternative can be used, namely seaweeds that promote the regeneration of plants.

In recent years, the use of natural seaweeds as fertilizer (Hong *et al*., 2007) has allowed for substitution in place of conventional synthetic fertilizer (Crouch and vanStaden, 1993). Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress, and enhanced post harvest shelf-life of perishable products (Beckett and van Staden1989; Norrie and Keathley, 2006) and extensively reviewed by Khan *et al*. (2009). Seaweed extracts are marketed as liquid fertilizers and biostimulants since they contain cytokinins (Durand *et al*., 2003; Stirk *et al*., 2003), auxins (Stirk *et al*., 2004), gibberellins (Wildgoose et al., 1978), betaines (Blunden et al., 1991; Wu et al., 1997), macronutrients (Ca, K, P) and micronutrients (Fe, Cu, Zn, B, Mn, Co, and Mo) (Khan *et al*., 2009), necessary for the development and growth of plants.

Therefore the development of a cost-effective and efficient protocol for mass propagation of high-quality seedlings through tissue culture by using seaweed extract as biostimulants instead of synthetic chemicals could help to overcome the above issue, since the seaweed extracts contains all basic nutrient requirements for plant growth. In this context, the present investigation was carried out to determine the efficiency of seaweed extract on plant regeneration of *W. somnifera.*

2. MATERIALS AND METHODS

2. 1. Preparation of in vitro callus of *Withania somnifera*

The nodes of three-month-old plants (*W. somnifera*) of the Indian cultivar were selected as mother plants. The explants were surface sterilized by using mercuric chloride solution (0.1 %) for 2-5 min and washed thoroughly with sterile distilled water. Explants were surface sterilized in detergent solution (Teepol-5 % v/v) for 10 minutes and by using mercuric chloride solution (0.1 %) for 5 min., each followed by two successive rinses in sterile distilled water.

The disinfected explants were taken for inoculation. Callus induction was achieved from nodal explants of *W. somnifera*in MS basal (Murashge and Skoog, 1962) medium with B5 vitamin (Gamborg et al., 1968). After preparing the media, it was incorporated with 2,4,-dichloro phenoxy acetic acid (0.5-3.0 mg/L).

The pH of the medium was adjusted to 5.8-6. The medium was distributed in 250 ml conical flask, autoclaved at 120 °C for 15 min at 15 lbs. The explants were then inoculated in the MS medium and kept under fluorescent illumination of about 3000 × lux and maintained at 25 °C. The maximum callus obtained in different concentration of 2,4,-d was used for shoot multiplication.

2. 2. Multiplication of shoots

To attain high-frequency shoot proliferation the callus thus obtained were cultured in MS basal (Murashge and Skoog, 1962) medium with B5 vitamin (Gamborg et al., 1968) supplemented with various concentrations seaweed extract *viz*. 10, 20, 40, 60, 80 and 100 %

were tried. Light and temperature conditions were maintained as described for the initial culture establishment system. The average numbers of shoots per explants were recorded. The seaweed extract used in the present study was of commercial one under the trade name of PYCOCOLIN and it is a kind gift of Dr. P. Anantharaman and Mr. Periyachamy, CAS in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai, Tamil nadu, India.

3. RESULTS

3. 1. Estabilishment of callus

The nodal explant cultured on MS-B5 basal medium without growth regulators thrived only for few days and shrived off. When the 2,4-D was added to the culture medium in increasing concentration from 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l, the explants showed callus formation (Table 1). The percentage of culture response from the nodal explant ranged from 44 to 80 and the maximum of callus formation was achieved in 3.0 mg/l. The proliferation of the callus from the explant was found after 2 weeks and it was harvested. The original callus was sub-cultured twice on fresh medium to obtain a mass of calli. After four week of culture there was a decline in the callus production and the callus became brown due to phenolic secretion.

Table 1. Effect of 2,4-D on callus induction of *Withania somnifera.*

S. No	Growth regulator 2,4-D (mg/l)	Culture showing response (%)	Basal callus
01	0.5	80	+
02	1.0	76	+
03	1.5	72	+
04	2.0	61	+
05	2.5	52	++
06	3.0	44	+++

+ : low; ++ : moderate; +++ : high

3. 2. Multiplication of shoots

MS media containing different concentration of seaweed extract (10, 20, 40, 60, 80 and 100 %) were tested individually for shoot induction. The number shoots produced by the callus in the MS medium supplemented with seaweed extract was ranged between 4.3 and 8.6 shoots. The medium supplemented with 40 % seaweed extract exhibited maximum number of shoots with about 8.6 shoots/ callus and 80 % seaweed extract exhibited 4.3 shoots/ callus. No shoot formation in 100 % seaweed concentration (Fig. 1).

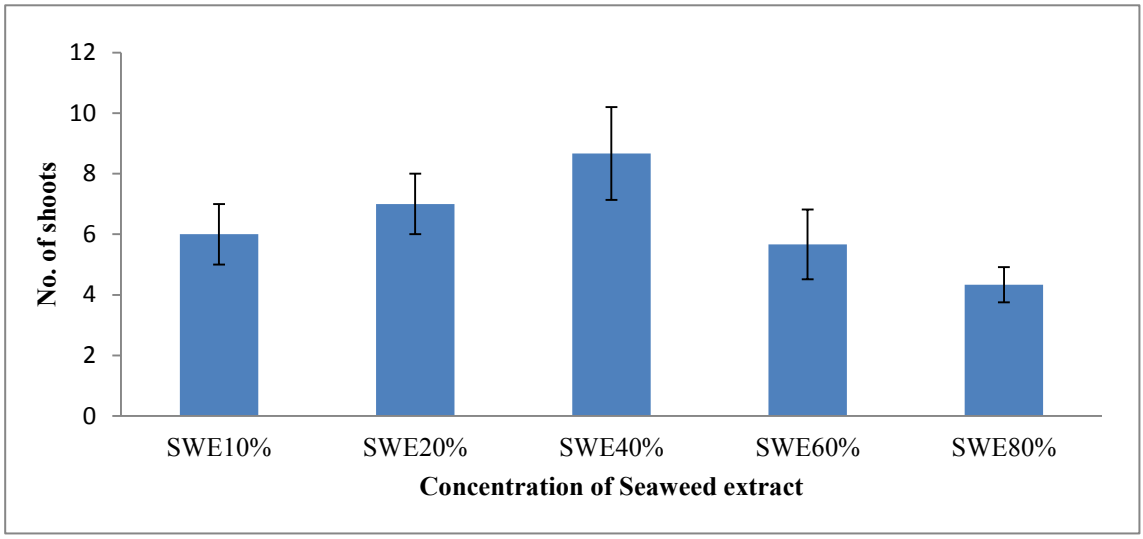

Fig. 1. Effect of Seaweed extract on shoot multiplication of *W. somnifera.*

4. DISCUSSION

In vitro propagation technology has sound and extensive potential for commercial rapid multiplication of medicinal plants and horticultural crops (Banerjee and Shrivastava, 20065D). *In vitro* culture methods through axillary bud multiplication using nodal segment have proved successful for quick propagation of number of medicinally important species such as *Santolina canescens* (Casado *et al*., 2002), *Bupleurum fruticosum* (Fraternale *et al*., 2002), *Rauvolfia tetraphylla* (Faisal *et al*., 2005). In the present study the percentage of culture response for callus initiation from the nodal explant ranged from 44 to 80 % and the maximum of callus formation was achieved in 3.0 mg/l. The callus initiation was observed in nodal segments explants within two weeks. Rout *et al*., (2011) reported out of 55 combinations of growth regulators tested, MS + 1.0 mg/l BA + 1.0 mg/l 2,4-D found to be most suitable for callus induction and proliferation regardless to leaf explantsof *W. somnifera* with the highest callus induction frequency of 86.4 %. According to Siddique et al., 2004 in W. somnifera 85 % of organogeniccallus was observed in nodal segments in MS medium containing 1.0 mg/l BAP and 2.0 mg/l Kin. De Silva and Senarath, (2009) reports callus initiation in leaf segments explants after a period of nine days' time with the mediun supplemented with 1.0 µM Kin, 4.5 µM BAP and 1.5 µM NAA. *In vitro* regeneration of endangered medicinal plant through callus is a prerequisite for the application of recent advanced techniques in plant improvement conservation. Nataraja and Sudhadevi (1984) reported that the epocotyl and cotyledonary leaf explants cultured on MS medium with 2,4-D and NAA, proliferated into a green compact mass of callus. Thus the present report confirms the earlier findings.

MS media containing different concentration of seaweed extract (10, 20, 40, 60, 80 and 100 %) were tested individually for shoot induction. The number of shoots produced by the callus in the MS medium supplemented with seaweed extract was ranged between 4.3 and 8.6 shoots/ callus. The medium supplemented with 40 % seaweed extract exhibited maximum number of shoots with about 8.6 shoots/callus and 80 % seaweed extract exhibited 4.3 shoots/callus. Similarly Vinoth et al, (2011) reported 30 % concentration of *Sargassum wightii* (Brown alga) *Gracilaria edulis* (Red alga) extract exhibited maximum number of

shoots with about 4.8 and 3.5 shoots per explant repectively. They also observed high frequency of shoot elongation in the medium supplemented with 30 % *G. edulis* (15.2 cm), and profuse rooting was observed in the medium supplemented with 50 % *S. wightii* of about 16.1 cm. Shoot elongation and rooting were observed in the medium supplemented with seaweed extracts. In the present study the higher concentration showed decreasing trends in shoot numbers.

Application of seaweed extracts enhanced shoot length and number of branches, root length and number of lateral roots at 20 % treatment (Kumar and Sahoo 2011). Seaweed extracts have growth-stimulating activities and are used as biostimulants in natural crop protection. It was reported that the seaweed extracts have promising plant growth regulators such as auxins, cytokinins, gibberellins, betains and major macronutrients and micronutrients that help in promoting the growth of various vegetables, fruits and other crops (Miers and Perry 1986; Taylor et al. 1990; Blunden et al. 1991).

Seaweed products are exploited in conventional vegetative propagation in many crop species (Crouch and van Staden 1991; Atzmon and van Staden 1994; Kowalski *et al*., 1999). It is common practice to apply auxins exogenously to enhance rooting in cuttings in certain species that are difficult to root. It has been observed that treating cuttings of some flowering plants like marigold (*Tagetus patula*) for about 18 h with 10 % SWC Kelpak increased the number and dry weight of roots (Crouch and van Staden 1991).

Similarly, Kelpak, when applied at a 1:100 dilution, increased the number of rooted cuttings and improved the vigor of the roots in difficult-to-root cuttings of *Pinus pinea* (Atzmon and van Staden1994). In another study, Leclerc et al, (2006) observed that foliar application of commercial liquid seaweed extract from Ascophyllumnodosum (Acadian Seaplants Limited), supplemented with BA and IBA, enhanced the number of propagules (crown divisions) per plant in the ornamental herbaceous perennial *Hemerocallis* sp. Similarly in the present study used inspite of plant growth hormones in tissues culture medium enhanced shoot production and minimized the cost of synthetic growth hormones.

On the other hand the commercial plant growth hormones were tested individually and in combination for their suitability to establish protocol for the organogenesis of *W. somnifera*. A maximum of 21.0 ±0.1 mean number of axillary shoots were obtained after two subcultures in presence of BA (1.0 mgl -1) and Kn (1.0 mgl -1) in MS medium (Tuhin and Biswajit, 2012). The effect of different combination of PGRs on shoot formation of *W. somnifera* on Murashige and Skoogs medium (MS),Schenk and Hilderbrant (HS) Gamborg B5 medium was studied and reported a maximum of 17 shoots at 2.0 mg/L of Bap+IAA in MS medium.

These values are higher than that of value obtained in the present study. Rout et al., (2011), tested different growth regulator combinations in augmentation with MS for shoot initiation and elongation, and reported, 2.0 mg/l BA + 1.0 mg/l NAA was the best eliciting a maximum of 82.3 % shoot induction with highest shoot number of 4.8 shoots/callus. But Soni et al., (2011) reported, maximum numbers of shoots (2.6) was produced on MS medium supplemented with 1.0mg/L BAP after 30 days of culture of *Withania somnifera*.

5. CONCLUSION

Hence, an efficient protocol was developed for micropropagation of multipurpose medicinally important endangered plant from nodal explants using seaweed extract as supplements could possibly be used for clonal propagation and conservation.

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