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EFFECT OF SUCROSE, pH AND AGAR ON *IN VITRO* SHOOT MULTIPLICATION OF *TERMINALIA ARJUNA*- A CARDIOTONIC TREE

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Terminalia arjuna (arjun) is most valuable medicinal tree species belonging to flowering plant family Combretaceae. The objective of the present study was to determine the effect of different factors on *in vitro* shoot multiplication of *Terminalia arjuna*. Different levels of sucrose, pH and agar were tested for growth and development of shoots during *in vitro* shoot multiplication. The media having 30 mg/l sucrose showed highest shoot multiplication in terms of shoot number and shoot length. Varied range of pH from 3.8-8.8 were tried to achieve maximum shoot multiplication. Among different levels of pH, the highest *in vitro* shoot multiplication was observed on the media adjusted to 5.8. The optimum concentration of agar for shoot multiplication was found to be 0.8%. The results presented here proved to be suitable for *in vitro* shoot multiplication of *Terminalia arjuna*.

Keywords: Agar; *In vitro* shoot multiplication; pH; Sucrose; *Terminalia arjuna*.

Introduction

Terminalia arjuna (arjun) is a large, handsome, evergreen, perennial and hardwood tree belonging to flowering plant family Combretaceae. It comprising around 250 species distributed in tropical region of the world[1]. Twelve species of *Terminalia* are native to India. In India, it is found in plenty throughout indo sub Himalayan tracts, Uttar Pradesh, Bihar, Jharkhand, Madhya Pradesh, Delhi, Orissa, Assam, Gujarat, Maharashtra, Tamil Nadu, West Bengal, Deccan region mainly along riverside, riverlets and ponds. It is a characteristic tree of dry tropical forests and riparian fringing forests and is one of the predominant species of Gir forests of Gujarat.

T. arjuna is an important medicinal tree as its bark is useful in many heart diseases. Apart from being the source of medicinal constituents, Arjun plays an important role in the sericulture industry[2]. The revival of interest of the wide spread belief that green medicine is healthier than synthetic products. Nowadays, there is manifold increase in medicinal plant based industries due to increase in the interest of use of medicinal plants. Propagation of *T. arjuna* is difficult by conventional methods, due to poor seed germination and seedling viability as well as inefficiency in rooting by cuttings and air-layering methods[3]. For commercial micropropagation of *T. arjuna* requires to develop protocol which will be able to produce multiple shoots in a shorter period of time and also technically feasible.

The most commonly used carbon source is sucrose and is act as an energy source for the proper growth and multiplication of *in vitro* shoots. Optimal pH of medium is required for proper growth and multiplication of *in vitro* shoot cultured. Most cultures required a gelling agent to act as a support for the plant tissue. Agar is most commonly used gelling agent for preparation of most of the tissue culture media. Therefore this study has been focused on three important factors of micropropagation of *T. arjuna*.

Material and Methods

The experiment was conducted at Plant Tissue Culture Laboratory, Forest Genetics Tree Breeding Division of Arid Forest Research Institute, Jodhpur. The cultures were established from nodal explants collected from lopped tree (10-20 years old) of *T. arjuna* situated at Ummaid garden, Jodhpur.

The *in vitro* proliferated shoots from nodal explants were further multiplied on modified MS medium (half strength of NH_4NO_3 and KNO_3) supplemented with 4.44 µM 6- Benzylamino purine (BAP) + 0.54 µM Naphthalene Acetic Acid (NAA) + additives (100 mg/l of Ascorbic acid, 50 mg/l of Citric acid, 50 mg/l of adenine sulphate and 25 mg/l of PVP). To study the best sucrose requirement for optimal shoot multiplication sucrose was added at concentration of 1-5% in medium. The pH of the medium was adjusted to 3.8-7.8 and medium was gelled with 0.5- 1.0% agar. Autoclaving of the medium was done at 15 psi for 15-20 minutes at 121°C temperature. All the cultures were kept in culture room at 25±2°C temperature

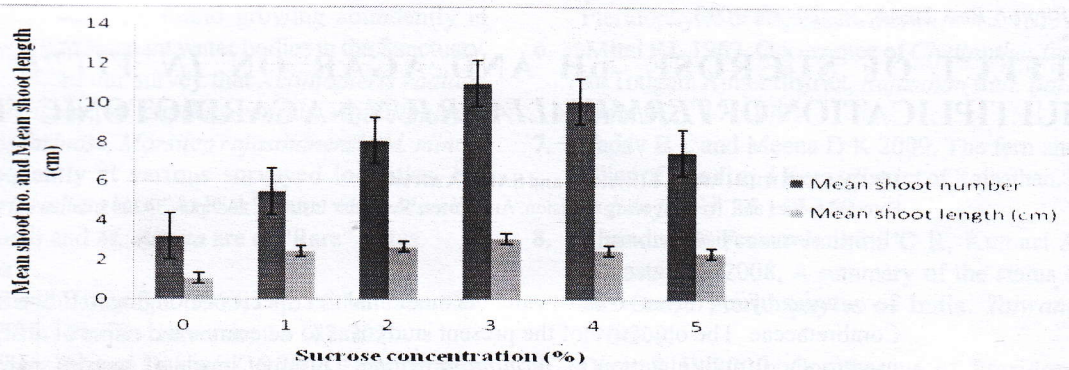

Fig.1. Effect of different concentration of sucrose on *in vitro* shoot multiplication of *T. arjuna.*

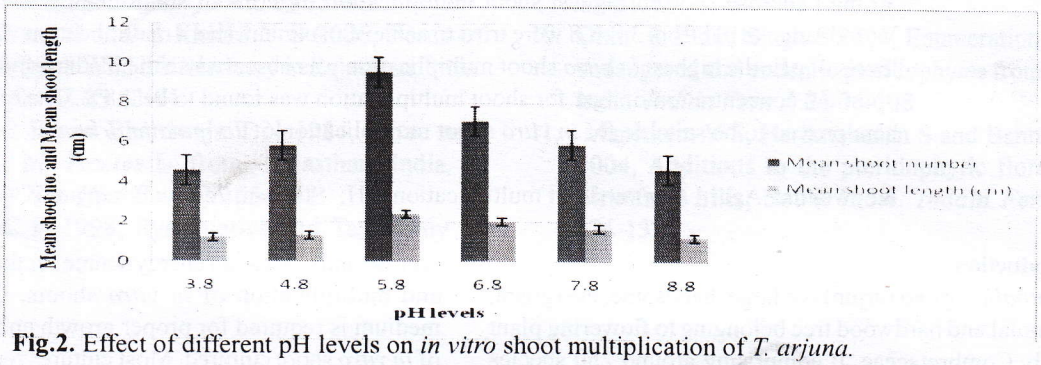

Fig.2. Effect of different pH levels on *in vitro* shoot multiplication of *T. arjuna.*

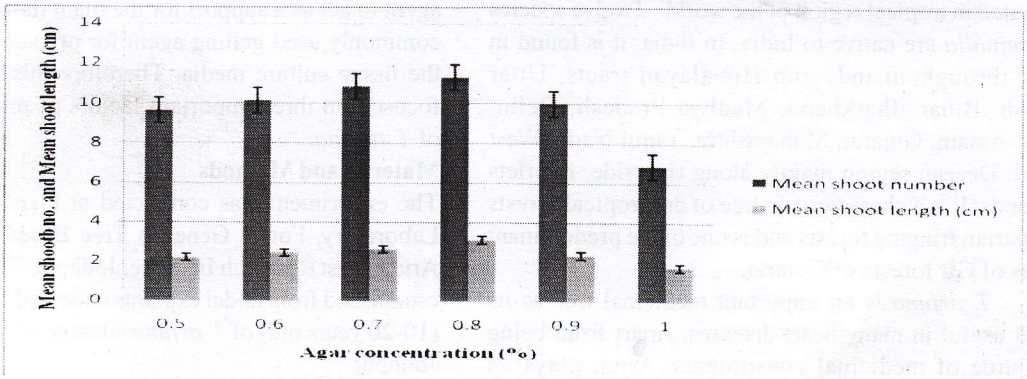

Fig.3. Effect of different concentration of agar on *in vitro* shoot multiplication of *T. arjuna.*

and 1600 lux light intensity for 16 h light and 8 h dark photoperiod using fluorescent cool white light produced by tubes and regulated by photoperiodic timer.

Statistical analysis- All the experiments were conducted with 15 replicate per treatment. Each experiment was repeated three times. Observations were recorded after 4 weeks. The results are expressed as mean ± SE of three experiments. The data were analyzed statistically using one way analysis of variance (ANOVA), and the significance difference between means were assessed by Duncan's multiple range test at $P< 0.05$.

Results and Discussion

Sucrose is readily assimilated and relatively stable and is act as an energy source for the proper growth and multiplication of *in vitro* shoots as the photosynthetic capability of the cultures was limited. Effect of different concentration of sucrose (1.0-5.0%) in MMS (modified MS) medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives was studied on *in vitro* shoot multiplication. The results revealed that 3.0% sucrose in the MMS medium was found to be optimal requirement for shoot multiplication. Maximum 11.02 shoots from

Table 1. Effect of sucrose, pH and agar concentration on *in vitro* shoot multiplication of *T. arjuna*. Shoot were cultured on MMS medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives.

	Sucrose concentration						pH of medium						Agar concentration					
concentration	©	1%	2%	3%	4%	5%	3.8	4.8	5.8	6.8	7.8	8.8	0.5%	0.6%	0.7%	0.8%	0.9%	1.0%
Mean shoot number	3.22 ± 0.10^{f}	5.50 ± 0.14^{e}	8.08 ± 0.20^{c}	11.02 ± 0.23^{a}	10.11 ± 0.18^{b}	7.50 ± 0.19^{d}	4.58 ± 0.16^{d}	5.83 ± 0.17^{c}	9.55 ± 0.18^{a}	7.08 ± 0.16^{b}	5.91 ± 0.18^{c}	4.66 ± 0.15^{d}	9.63 ± 0.22^{b}	10.11 ± 0.21^{b}	10.83 ± 0.22^{a}	11.30 ± 0.24^{a}	9.97 ± 0.20^{b}	6.83 ± 0.23^{c}
Mean shoot length (cm)	1.10 ± 0.03^{d}	2.47 ± 0.07^{c}	2.69 ± 0.06^{b}	3.09 ± 0.07^{a}	2.51 ± 0.05^{c}	2.39 ± 0.05^{c}	1.19 ± 0.03^{d}	1.30 ± 0.03^{d}	2.38 ± 0.04^{a}	1.98 ± 0.07^{b}	1.65 ± 0.04^{c}	1.21 ± 0.04^{d}	2.18 ± 0.04^{d}	2.43 ± 0.05^{c}	2.61 ± 0.04^{b}	3.08 ± 0.06^{a}	2.30 ± 0.04^{cd}	1.70 ± 0.05^{e}

© = Control. Mean value followed by the same latter are not significantly different according to Duncan's multiple range test at P<0.05.

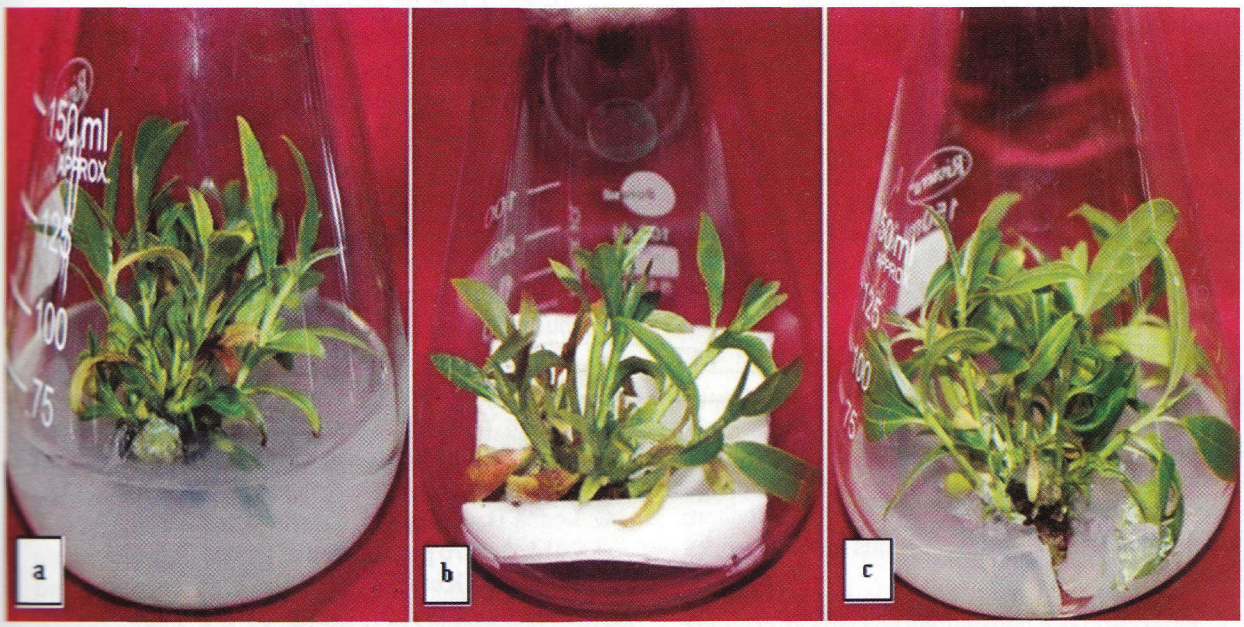

Fig.4. *In vitro* shoot multiplication of *T. arjuna* affected by Sucrose, pH and Agar concentration.
a. *In vitro* shoot multiplication on MMS medium containing 3% sucrose; b. *In vitro* shoot multiplication at 5.8 pH of the MMS medium; c. *In vitro* shoot multiplication on MMS medium supplemented with 0.8% Agar.

propagule of three shoots were obtained in four weeks (Fig. 4a). At higher concentration of sucrose (4.0-5.0%), no further increase in shoot multiplication rate was obtained. At lower concentration of sucrose (1.0-2.0%), reduced multiplication rate was obtained (Fig. 1, Table-1). On sucrose free medium multiplication rate sharply declined with yellowing of leaves in the subcultured shoots. The results of the present investigation are similar with the reports of many workers who used 3% sucrose as a source of carbohydrate for shoot multiplication of different tree species like *Tona ciliate*[4], *Chrysanthemum morifolium*[5], *Rauvolfia tetraphylla*[6], *Bambusa nutans*[7] and *Amygdalus communis*[8].

The pH of the medium played a key role for

optimum uptake of nutrient and organic salts required for proper growth and multiplication of *in vitro* shoot cultures. For this, effect of different pH values was tested for *in vitro* shoot multiplication. Varied range of pH from 3.8-8.8 were tried in liquid MMS medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives. In acidic medium conditions, *in vitro* shoots developed were small and condensed. At increase pH levels (5.8-8.8) clear distinct shoots were obtained. However, in strong acidic and basic medium the shoot multiplication drastically reduced (Fig.2, Table-1). Low or high pH levels than 5.5 caused serious abnormalities, producing shorter shoots with narrow, curled and sharp-pointed leaves in *Amygdalus communis*[9]. At 5.8 pH of MMS medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives, optimum shoot multiplication of 9.55 shoots per propagule of three shoots with sizeable shoots (2.38 cm) was obtained (Fig.4b). These results compare favorably with those of other worker in *Azadirachta indica*[10], *Citrus aurantifolia*[11] and *Cassia angustifolia*[12].

Agar-agar (0.5-1.0%) was added in MMS medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives to study the effect of agar concentration for *in vitro* shoot multiplication. On medium gelled with 0.8% agar-agar, maximum 11.30 shoots with 3.08 cm shoot length were obtained (Fig.4c). A decline trend of shoot multiplication was observed with increasing and decreasing concentration of agar-agar (Fig.3, Table-1). The results of the present investigation are similar with the reports of many workers who used 0.8% agar as gelling agent in *Bambusa tulda*[13], *Ephedra foliate*[14] and Capparis decidua[15].

In conclusion, plant tissue culture provides alternate means for mass production of selected tree species. Sucrose, pH and agar are three important factors that significantly affect the multiplication of *T. arjuna*. By optimizing these factors, multiple shoots with short period of time can be obtained *in vitro*.

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