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Original Article

EFFECT OF SUCROSE AND MEDIA STRENGTH ON *IN VITRO* MULTIPLICATION IN *SWERTIA CHIRATA BUCH.-HAM EX WALL: AN ENDANGERED MEDICINAL HERB*

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ABSTRACT

Objective: The present study was performed to investigate the role of varying concentrations of carbohydrate source and strengths of nutrient medium in growth and development of *in vitro* shoots of *Swertia chirata*-an endangered medicinal plant.

Methods: MS medium supplemented with 6-Benzylaminopurine (4.44 µM), Indole-3 acetic acid (2.85 µM) and Adenine sulphate (271.45 µM) was used to test the efficiency of of sucrose at concentrations of 1-5% and of media strength varying from full to one-fourth. The data were analysed using analysis of variance (ANOVA) of Completely Randomized Design (CRD) in GenStat 5 Edition 3.2 for PC/Windows NT **(**Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station))

Results: Observations on axillary shoot multiplication indicated that sucrose at a concentration of 3% and MS medium in its full strength proved to be most optimal for *in vitro* culture multiplication. On this medium combination mean number of 11.80 shoots (after 4 w) and 18.50 shoots (after 8 w) could be obtained On sucrose free medium the shoots exhibited necrosis while at lower concentrations of 1-2% sucrose, the shoots developed were thin and unsuitable for further growth *in vitro.* At higher levels of sucrose in the medium, the shoots became thick and stunted. Similarly, reduction in medium strength resulted in a decline in shoot number and shoot length to an average of 6.50 shoots (1.33 cm mean length) on half strength medium and 5.60 shoots (0.88 cm mean length) on one-fourth strength; as observed after 4 w.

Conclusion: The experimental findings suggest that any decline from the standard had a significant effect on the number, size and overall health of shoots developed *in vitro*. The conditions so standardized augment the production of healthy shoots that shall aid in subsequent rooting and survival after transplantation of tissue-culture raised plantlets.

Keywords: *Swertia chirata, In vitro* propagation, Medicinal plant, Shoot length, Shoot multiplication

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INTRODUCTION

Swertia chirata is a native of temperate Himalaya and widely acknowledged as a medicinal herb to cure chronic fever, anaemia, blood, liver, lung and skin disorders [1-4]. The bitter active ingredients of the plant viz. amarogentin, xanthones, iridoid glycosides, mangiferin and C-glucoflavones [5-7] account for its wide range of medicinal and curative powers. The plant, therefore, enjoys a good national and international market. However, *Swertia chirata* has now been categorized as critically endangered [8-11] owing to unscientific collection practices from the wild; aided by poor seed germination rate in nature (only 2-4%) and low seed viability [12-14]. Consequently, development of alternative propagation strategies for this important medicinal plant becomes the call of the hour. Some studies have already been performed to develop large-scale propagation protocol for *S. chirata* via tissue culture technology. However, there is a lacuna in studies to standardize different physical parameters involved in the propagation of plant *in vitro.* The present work was undertaken to investigate the effect of different carbohydrate concentrations and medium strength on *in vitro* shoot multiplication of *S. chirata*. The findings shall ensure optimal utilization of resources in establishing a standardized system for maximal production of healthy plantlets of *Swertia chirata*.

MATERIALS AND METHODS

Chemicals and reagents

Standard analytical grade chemicals of Merck, India, and Hi-media laboratories, India were used in the present study. The culture media comprised of inorganic salts, organic compounds, amino acids, plant growth regulators, carbohydrate source and gelling agents. All chemicals used for stock preparation were of analytical grade. All major and minor salts components of MS medium used were of Hi-media laboratories, India. Plant growth regulators, adjuvants, sucrose (carbohydrate source), agar (gelling agent) used were also of Hi-media laboratories, India. Auxins, cytokinins used were dissolved in dilute 1.0 N NaOH or 1.0 N HCl.

General methodology

In vitro cultures established via axillary bud proliferation and multiplying on MS medium containing BAP (4.44 µM)+IAA (2.85 µM)+Ads (271.45 µM) (Pant *et al.* 2010) were used to assess the effect of varying concentrations of sucrose and media strength on shoot multiplication in *Swertia chirata.* For the purpose, shoots were separated from established cultures and transferred to MS medium fortified with PGRs and gelled with 0.7% agar. Sucrose was used as the carbohydrate source at concentrations of 1-5% in the multiplication medium while MS medium strength was tried at three levels: (1/4 X, 1/2 X, 1X) to standardize conditions for best results of *in vitro* multiplication. In all experiments, the pH of the medium was adjusted to 5.8 prior to autoclaving the medium at 121 °C and 1.0 x 10^5 Pa for 15 min.

Data collection and analysis

Observations on average shoot number and average shoot length were recorded after an interval of 4 w and 8 w. All experiments were repeated thrice. Each treatment consisted of minimum ten replicates. The data were analysed using analysis of variance (ANOVA) of Completely Randomized Design (CRD) in GenStat 5 Edition 3.2 for PC/Windows NT (Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)). The significance level

was determined at 5 % (p<= 0.05), 1% (p<= 0.01) and 0.1% (p<= 0.001). Mean values of treatments were compared with Least Significance Difference (LSD). Treatments whose difference was less than LSD were considered to be the same while those with the difference more than LSD were considered to be significantly different.

Culture conditions

The cultures were incubated in a culture room at 25±1°C temperature and 60-65% relative humidity under a 16/8 h (light/dark) photoperiod with light supplied by cool-white fluorescent tubes (Philips, India) at an intensity of 2500 lux.

RESULTS AND DISCUSSION

In the present study, significant differences were observed in all parameters of shoot multiplication in all the treatments tried. Plantlets growing under *in vitro* conditions have limited accessibility to CO_2 inside the vessel [16]. Therefore, sugar is supplemented as a carbon source to maintain an adequate supply of carbon for *in vitro* multiplication and growth of plant cell, tissue, and organs or whole plantlets. Sucrose is the most commonly used source of carbohydrates, having both metabolic and osmotic functions. It provides the carbon precursors for structural and functional components [17], meets the energy demands for growth and physiological functions, maintains the osmotic potential of cells and water conservation [16, 18].

In the present study, sucrose in the medium was found to be indispensable for shoot multiplication. On sucrose-free medium, the *in vitro* shoots initially multiplied slowly. However, within few weeks, the leaves started becoming pale and eventually necrosed. At lower concentrations of sucrose (1-2%) an average of 6.90 shoots after 4 w and 10.20-10.50 shoots after 8 w could be obtained [table 1]. The shoots so developed were stunted and an average of only 1.53 cm-1.61 cm shoot length after 4 w and 1.85 cm-2.24 cm after 8 w was attained [fig. 1 A, B]. Contrasting to these observations, successful multiplication with 2% sucrose has been reported in Chinese medicinal plants [19] and *Bacopa monniera* [20]. Similar observations were also reported in *Swertia chirata* [21] while working on *in vitro* propagation using seedling derived shoot tip explants. Plantlets growing on sucrose supplemented media have been known to exhibit reduced photosynthesis, probably due to the presence of sufficient energy source (sugars) for other metabolic activities [22-24]. Thus, a decrease in sucrose levels in the medium could be beneficial for *in vitro* plantlets. However, in our study absence of sucrose in the multiplication medium hampered *in vitro* multiplication with shoots becoming dead after a period of 4 w. At 1% sucrose, thin shoots and leaves developed which were not suitable for further sub-culturing while at 2% concentration the average number of shoots developed and average shoot length was significantly less than the results obtained at 3% concentration.

A report on bamboo showed that increased level of sucrose at 3-4% did not affect shoot number but caused albinism [25]. No such observations were made in the present study and sucrose at concentrations 3-4% produced healthy shoots with the development of normal sized leaves. However, with increased levels of sucrose (5%) shoots became thick and stunted shoots along with declined multiplication [fig. 1 E]. Sucrose at 3% level was found to be the most optimal and essential for the development and growth of shoots [fig. 1C]. On this combination, an average of 11.80 shoots with average shoot length 1.94 cm after a period of 4 w, and 18.50 shoots with average shoot length 2.55 cm after 8 w was recorded. It has been reported that sucrose supplied at a concentration of 3% in the medium increases the photosynthetic ability, thereby improving survival of plantlets [26]. The results of the present investigations are also compatible with previous reports in other medicinal plants viz. *Gentian kurroo* [27], *Gentiana sps.* [28], *Ocimum gratissimum* [29], *Bacopa monnierri* [30], *Gentiana scabra* [31], *Cassia angustifolia* [32]. Henceforth, sucrose at 3% concentration was considered to be most efficient for *in vitro* shoot multiplication of *S. chirata*. The observations are in corroboration with previous studies on *Swertia chirata* reporting the efficacy of 3% sucrose for *in vitro* multiplication via nodal and shoot tip explants [33-36].

The inorganic salt formulation of MS medium [37] is widely used in plant tissue culture studies for providing basic nutrition to the growing tissues supplemented by plant growth regulators or natural additives for stimulated growth. MS medium is a high salt medium with high levels of nitrogen, potassium and some of the micronutrients, particularly boron and manganese [38]. This high salt content often makes it unsuitable for optimal development of plantlets *in vitro*; resulting in the need to modify the media strength. In the present study, the effect of media concentrations on *in vitro* shoot multiplication was studied, and the highly significant difference was observed among the treatments tried [table 2]. It was observed that the best results of shoot multiplication were obtained on full strength MS medium. Reduction of salt strength in the medium resulted in a proportional decline in shoot development. The mean number of shoots multiplied and average length of shoots decreased from 11.80 (1.94 cm) in 1X to 6.50 (1.33 cm) in 1/2X and 5.60 (0.88 cm) in 1/4X medium after 4 w and from 18.50 (2.55 cm) in 1X to 12.20 (1.88 cm) in 1/2X and 9.20 (1.23 cm) in 1/4X medium. Thus, MS medium in its full strength was found to be most effective for optimal *in vitro* shoot multiplication in *S. chirata* (fig. 2 A, B, C). A direct association between medium strength and the number and length of shoots could be associated with the fact that altering media strength changes the concentration of essential nutrients required for tissue organogenesis.

As a result, the amount of nutrients in culture media also varies for different plant species and genotype and needs to be standardized for protocol development. Our results are in corroboration with previous studies on medicinal plants where full strength MS medium has proven to be efficient for *in vitro* shoot multiplication [34, 35, 39].

CONCLUSION

The present results indicate the importance of maintaining appropriate levels of carbohydrate source and nutrient strength in culture medium used for *in vitro* propagation of an important and endangered medicinal plant *Swertia chirata.* Any deviation from the optimal level is suggested to cause a deterioration in healthy shoot development and subsequently hampered *in vitro* rooting and transplantation.

ABBREVIATION

Murashige and Skoog's (1962) (MS), 6-Benzylaminopurine (BAP), indole-3 acetic acid (IAA), adenine sulphate (Ads), medium strength (X), plant growth regulator (PGR)

AUTHORS CONTRIBUTIONS

All the experiments were done by the first author. Second and third author supervised the work, and all authors were involved in designing of the experiment and preparation of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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