December 2007 Volume 1 Issue 4

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 1(4), 2007 [156-160]

Influence of sucrose on morphogenetic changes of four rare and endangered ferns of western ghats, South India

M.Johnson*#, V.S.Manickam
Centre for Biodiversity and Biotechnology, St. Xavier's college (Autonomous), Palayamkottai, India,
Tel: +251 582 20 75 45; Fax: + 251 582 20 20 25
E-mail: biojohnson@sify.com
#Biology Department, Bahir Dar University, Bahir Dar, P. O.Box. 1501, (ETHIOPIA)
Received: 8th October, 2007 ; Accepted: 13th October, 2007

ABSTRACT

The present study was intended to study the effect of sucrose on spore germination, gametophyte development and sporophyte formation. The present study revealed that each and every plant requires different nutrient composition and sucrose for their development. The spore germination was inhibited by the sucrose supplemented media, basal media showed the spore germination. The highest percentage (82.6±0.72) of sporophyte emergence was observed in KC medium supplemented with 1% sucrose *(Cheilanthes viridis* (Forssk.) Swartz)., (83.8±1.31) of sporeling emergence was observed in Mi medium supplemented with 1% sucrose *(Phlebodium aureum* L.), KC augmented with 3% sucrose and KN with all the concentrations of sucrose showed sporeling emergence *Pronephrium triphyllum* (Sw.) Holttum, and KN medium supplemented with 1% sucrose showed high percentage (78.3±1.21) sporeling formation in *Sphaerostephanos unitus* (L.) (Holttum). The basal medium promotes the gametophyte formation compared to the sporophytes formation. In *Sphaerostephanos unitus* the apogamous sporophytes formation was stimulated by the sucrose supplementation, 1% sucrose supplemented media inoculated presexual gametophytes induced the apogamous sporophytes formation.

KEYWORDS

Gametophyte;
Sporophyte;
Apogamous;
in vitro;
Sucrose

INTRODUCTION

Micropropagation of any plant material depends on the appropriateness of the nutrients supplied in the culture medium. For *in vitro* culture, water, macro and micronutrients and sugar are needed for plant growth and development. Any change in the medium results in variation in response of the explants. Differentiation and morphogenesis directly depend on the nutrients and growth regulators in the nutrient milieu. Not only nutrients and PGRs, but also various other factors affecting plant regeneration from excised cells or tissues/callus have been studied[6]. *In vitro* spore germination is the best applied and commercially exploited technology in fern species[5]. The advantages of *in vitro* spore germination of ferns in developmental and differentiation studies are described by many authors[12,13,14,10]. Sugar is a very important component in any nutrient medium and

its addition is essential for *in vitro* growth and development. The sugar at a particular concentration directly depends on the type and age of growth material/explants. Sucrose was found to be the best universal source of carbon in plant micropropagation medium followed by glucose, maltose, fructose and raffinose. The concentrations of sucrose play a vital role in the developmental/ morphogenesis of plants particularly in pteridophytes. Literature available vouch for the role of sugar on gametophyte development and sporophytes induction[11,1,2,10,3]. By manipulating the sucrose concentration in the culture medium, production of haploid, diploid and tetraploid sporophytes as well as gametophytes was demonstrated[8]. Based on this background the present study was initiated to study the impacts of sucrose concentrations on the development and morphogenetic activities of *Cheilanthes viridis* (Forssk.) Swartz, *Phlebodium aureum* L., *Pronephrium triphyllum* (Sw.) Holttum, *Sphaerostephanos unitus* (L.) (Holttum, rare and endangered ferns of Western Ghats, South India. In addition the present study emphasized on influence of sucrose on apogamous sporophytes production also.

Abbreviations

KC-Knudson C medium; KN-Knop's medium; Mi-Mitra medium; Mo-Moore's medium; MS-Murashige; Skoog's medium.

MATERIALS AND METHODS

The spores of *Cheilanthes viridis* (Forssk.) Swartz, *Phlebodium aureum* L., *Pronephrium triphyllum* (Sw.) Holttum, *Sphaerostephanos unitus* (L.) (Holttum) were collected from the fertile fronds and passed through nylon mesh (40µm) to remove the sporangial wall materials and clean spores were collected and used for culture initiation. They were surface sterilized with 0.1% $HgCl_2$+0.1% sodium lauryl sulfate solution for 3-5min. and washed with sterile distilled water for 15min. For spore germination, gametophyte multiplication and sporophyte formation, the spores were cultured on liquid and agar gelled with various media with various sucrose concentrations. The cultures were incubated under 12h photoperiod/day at $25^0C \pm 2^0C$ C and observations were made periodically and data recorded. After the inoculation, the cultures were incubated at $25^0C \pm 2^0C$ under 70% relative humidity and 12h photoperiod/day in a culture room. All the cultures were kept in 1200-1500 lux light intensity provided by cool white fluorescent tubes (Phillips India Ltd, Mumbai). The spore derived gametophytes were subcultured on different media with different concentrations of sucrose for gametophyte multiplication and sporophyte formations.

RESULTS

Spore germination

The spores of *Cheilanthes viridis, Phlebodium aureum* and *Pronephrium triphyllum*, were failed to germinate on sucrose supplemented media. *Sphaerostephanos unitus* spores were germinated 1% sucrose supplemented media, above 1% sucrose concentration were failed to germinate.

Gametophyte and sporophyte development

Cheilanthes viridis (Forssk.)

In vitro raised gametophytes from basal media were transferred to appropriate media with different concentrations of sucrose. The highest sporophyte(82.6 ±0.83%) emergence was observed in KC medium supplemented with 1% sucrose. The optimal concentration of sucrose ranged from 1-3%; at the concentration exceeding 3% the sporophyte emergence was reduced. The gametophyte turned pale yellowish green in colour with occasional in callus formation. The maximum length(3.03±1.1) of the sporophyte was observed in KC medium supplemented with 1% sucrose. The maximum number(38±1.53) of sporophytes was observed in KC medium supplemented with 3% sucrose. Both number and length of the sporophyte decreased in concentrations exceeding 3%. The minimum length of the sporophyte(1.25 to 0.31) was observed in KC medium supplemented with 5% sucrose. The highest percentage of callus formation(61.4±1.17) was observed in KC medium supplemented with 5% sucrose (TABLE 1).

Phlebodium aureum *L.*

The basal raised gametophytes of were transferred to Mi, Moore, KC and KN media supplemented with

Regular Paper

TABLE 1: Influence of Sucrose on alternation of generations in *C.viridis, P.aureum, P.triphyllum and S.unitus*

Name of the species	Medium with different sucrose concentration (%)	% of gametophyte multiplication ± S.D.	% of sporophyte formation ± S.D.	Mean no. of croziers (cms) ±S.D	Mean length of croziers (cms) ±S.D.	% of callus formation ± S.D.
C.viridis	KC - 1%	27.3±0.91	82.6 ± 0.83	30.3 ± 1.3	8.03 ±1.1	NIL
	KC - 2%	32.7±0.78	80.3 ±0.72	33.4 ± 1.64	7.27 ± 0.78	NIL
	KC - 3%	24.5±0.67	80.1 ±0.63	38.7 ± 1.53	5.09 ± 0.71	32.3 ± 0.81
	KC - 4%	11.8±0.79	55.2 ±0.78	22.3 ± 1.19	3.48±0.44	50.7 ±0.88
	KC - 5%	Nil	51.3 ± 0.81	18.33 ± 1.32	1.82 ± 1.24	61.4 ±1.17
	KC - Basal	78.3±0.81	91.3 ± 1.21	7.1 ± 0.31	1.72 ± 0.57	Nil
P.aureum	Mi - 1%	40.3±1.21	83.8 ±1.31	4.8 ±0.81	1.4 ±0.81	-
	Mi - 2%	52.8 ±0.81	29.8 ±0.81	2.1 ±0.71	1.1±0.87	20.3 ±1.24
	Mi - 3%	48.1±0.34	11.3 ± 0.84	0.9 ±0.31	0.92 ± 1.01	48.3 ±1.31
	Mi - 4%	40.9±0.43	-	-	-	60.3 ±1.14
	Mi - 5%	24.1 ±1.34	-	-	-	68.3 ±0.84
	Mi - Basal	88.3 ± 1.41	38.4 ± 1.21	1.8 ± 1.14	1.38 ± 0.63	Nil
P.triphyllum	KN - 1%	55.3 ±0.81	11.8 ± 0.34	4.5 ±0.63	1.1 ±0.29	24.3 ±1.21
	KN - 2%	50.3 ±1.13	52.1 ±0.81	7.8±0.5	1.5 ±0.61	30.1 ±1.3
	KN - 3%	38.3 ±0.83	34.3 ±1.04	3.81 ±0.66	1.1 ±0.13	60.3 ±0.93
	KN - 4%	32.3 ±1.21	23.8 ±0.71	2.7 ± 0.49	0.99±0.29	65.1 ±0.73
	KN - 5%	25.3 ±0.34	19.3 ±0.53	2.0 ±0.816	0.83±0.2	65.3 ±0.83
	KN - Basal	67.3±1.31/72.3±1.08	Nil	Nil	Nil	Nil
S.unitus	KN - Basal	74.8 ±1.31	76.8 ±1.41	3.2 ±1.13	6.3 ±1.38	Nil
	KN - 1%	38.8 ±1.13	78.3 ±1.21	43.6±1.61	2.39 ±0.76	Nil
	KN - 2%	31.3 ±0.83	61.1 ±1.13	42.5 ± 1.8	2.49 ±1.05	Nil
	KN - 3%	28.8 ±1.03	51.3 ± 0.83	27.2±0.9	1.77±0.78	28.3 ±1.21
	KN - 4%	26.5 ±1.3	37.5 ± 1.31	20.8 ±0.61	0.78 ±0.13	43.8 ± 1.43
	KN - 5%	18.5 ±1.34	11.1 ±0.31	7.3 ±0.81	0.6 ±0.21	53.1 ±1.21
Pre Sexual	KN - Basal	68.6 ± 1.36	Nil	Nil	Nil	Nil
	KN - 1%	40.3±1.34	74.5±1.43	18.3 ±1.31	2.8 ±1.1	Nil
	KN - 2%	36.8 ±1.38	65.3 ±1.38	12.6 ±1.34	2.43 ±1.6	Nil
	KN - 3%	30.8 ±1.43	48.3 ±1.21	9.8 ±1.1	1.88±1.3	25.3 ±1.21
	KN - 4%	25.8 ±1.48	38.3 ±1.13	8±0.93	1.73 ±1.3	52.1 ±1.37
	KN - 5%	16.3 ±1.48	18.3 ±1.21	7 ±1.1	1.63 ±1.3	58.3 ±1.44

different concentrations of sucrose. Highest sporophyte emergence (83.8±1.31) was observed in Mi medium supplemented with 1% sucrose and very low percentage (11.3±0.84) was observed in Mi medium supplemented with 3% sucrose. Sporophyte emergence was not observed in high concentration of sucrose (4 and 5%) in Mi medium and in all the concentrations of sucrose in all the other media. Callus formation (48.3) was observed in all the media supplemented with 3% sucrose and at concentrations exceeding the level, sucrose caused browning of tissue with formation of yellowish brown callus [4% (60.3±1.14) and 5% (68.3±0.84)] (TABLE 1).

***Pronephrium triphyllum* (Sw.) Holttum**

The Knudson C medium supplemented with 3% sucrose showed the highest percentage (42.3±1.38) of sporophyte emergence. The KC medium augmented with other sucrose concentrations failed to produce sporophyte. But in KN medium, all the concentrations induced sporelings, the percentage of sporophyte emergence was varied. The highest percentage(52.1±0.81) of sporophyte emergence was observed in KN basal medium supplemented with 2% sucrose , Followed by 3% sucrose in the same medium (34.3±1.04). High concentration of sucrose(4% and 5%) induced high percentage of callus formation. The calli were yellowish brown in colour. The gametophyte multiplication percentage (68.3±1.23) was high in KC medium supplemented with 1% sucrose. The number (7.8±0.5)

of sporophytes was high in KN medium supplemented with 2% sucrose (TABLE 1).

***Sphaerostephanos unitus* (L.) (Holttum)**

The Knudson C medium supplemented with 3% sucrose was induced sporophytes. Other concentrations of sucrose failed to produce sporophytes. KN medium supplemented with 1% sucrose induced highest percentage of sporophyte formation (78.3±1.21). Lowest frequency (11.1±0.31) was observed in KN medium supplemented with 5% sucrose. KC medium supplemented with various sucrose concentrations failed to produce callus. KN medium supplemented with sucrose concentrations exceeding 2% induced the callusing to varied extension(TABLE 1). As part of the experiment, the pre- sexual stage gametophytes / protonema (before sex organ formation) were transferred to different concentrations of sucrose for sporophyte emergence. KN medium supplemented with 1% sucrose induced high frequency(74.5±1.43) formation of agamosporous/apogamy sporophyte. Sucrose concentration 1-3% induced sporophyte formation concentrations of sucrose exceeding 3% inhibited sporophyte production, but induced callus formation (TABLE 1).

DISCUSSIONS

Normally for the culture of cells, tissues, or organs, it is necessary to incorporate a carbon source into the medium. Sucrose is almost universally used for micropropagation purposes. In the present investigation also, various nutrient media were supplemented with different sucrose concentrations for spore germination and the results indicated lack of spore germination in sucrose-supplemented media. In controversy to the present observations[3,10] reported the significant increase in spore germination of fern species by addition of sucrose in the medium. Generally growth and development increases with increased sugar concentration until an optimum is reached and then decreases at very high concentration. Camloh[2], observed the stimulatory effect on gametophyte development; in the present study also similar enhanced developments were observed. In controversy, Douglos[4] observed the inhibitory effect in *Anemia* and *Pteridium* gametophytes. The optimal concentrations of sucrose favored sporeling emergence and high concentration induced callus formation. All the sucrose supplemented media were not showed the sporophytes formations, the KC medium supplemented with 3% sucrose enhanced the sporophye formation for the selected species, other concentration were failed to express the sporophytes formation except *C. viridis*. The KN media was suitable for Thelypetridaceae members viz., *P. triphyllum* and *S. unitus* showed the sporophytes formation in KN augmented with different sucrose concentration. In *P.aureum* also, the sporophyte formation was observed in all concentration. Generally, the highest percentage of sporophytes observations observed in 1% supplemented culture tubes. The present study results were directly consonance with Thentz and Moncousin[11] observations in *P.bifurcatum* gametophytes; they also observed the highest percentage of sporophytes formation in 1-2% sucrose supplemented media. In controversy to the present study observations, Whittier[12] observed that the older gametophytes grew very well in 4% sucrose while in the present study higher concentrations were promoted the callus formation. Miller and Miller[9] and Kato[7] observed the highest sporophytes formation in 2% sucrose supplemented media, in addition they noted the importance of age of explants also, they observed the highest percentage of sporophytes formation in younger tissues. Sucrose (above 3%) was an essential ingredient in the medium to induce callus formation. In the present study, KC, Mi and KN supplemented with 4% and 5% sucrose induced high percentage of callus formation; they failed to enhance the sporophytes formation and gametophyte multiplication. In *P.aureum* and *S.unitus*, the pre-sexual stage (before sex organ development) gametophytes were induced to develop into sporophytes without any sex organ. Thus the production of sporophytes was solely dependent on the presence of sucrose. The basal medium (without sucrose) failed to produce sporophyte in *S.unitus* and *P.aureum*. Sucrose plays a key role in controlling the differentiation in different phases in fern system. Low or no sucrose concentration in the medium favored gametophyte formation and multiplication, while higher concentration resulted in the shift towards sporophyte and callus formation. Fundamentally, the sporophytic and gametophytic tissues are identical, posessing all the coded information needed to produce gametophytes or sporo-

Regular Paper

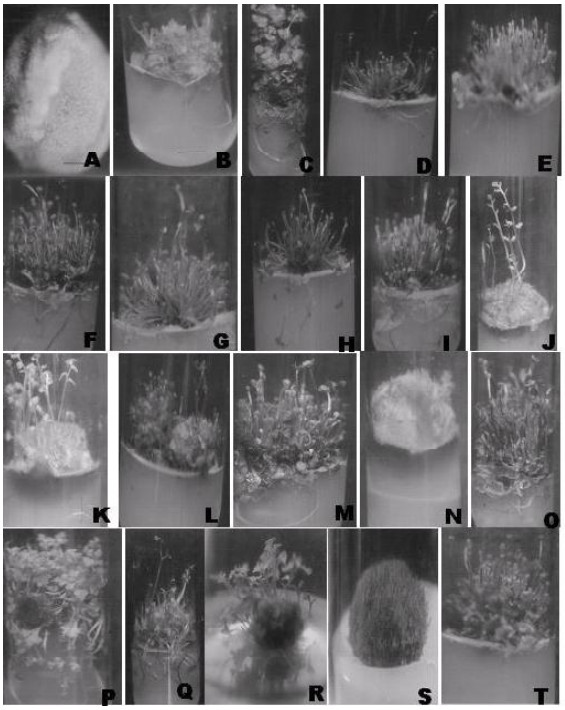

Figure 1

phytes depending upon the precise nutritive environment. In the present study we observed that, all the four selected species produced sporophytes in low sucrose (1%) augmented media, above 3 % showed the unfavorable condition they induced the callus formation, it lengthen the life cycle of the species. From the observation we suggest that the optimal condition for these four rare and endangered species is 1 to 3% sucrose supplemented media. The present study describes the optimal requirements for *in vitro* gametophyte multiplication, sporophytes formation and callus formation of *C.viridis*, *P.aureum*, *P.triphyllum* and *S.unitus*. The developed protocol may enhance the large scale multiplication of the rare and endangered species and help in the promotion of its cultivation and compensation of its loss in the wild.

ACKNOWLEDGMENT

This work was supported by Ministry of Environment of Forests, Government of India, New Delhi, India.

REFERENCES

[1] M.Camloh, N.Gogala; Scientia Horticulturae, **51**, 343-346 **(1992)**.
[2] M.Camloh; Amer.Fern.J., **83**, 124-133 **(1993)**.
[3] H.C.Chang, D.C.Agrawal, C.L.Kuo et al; *In Vitro* Cellular & Developmental Biology – Plant DOl 10.1007/s11627-007-9037-6, **(2007)**.
[4] G.E.Douglos; An investigation into the growth, development and ultra structure of fern gametophytes in existing and novel culture systems, University of Manchester, UK, PhD Thesis, **(1994)**.
[5] M.F.Fay; Biodiversity and Conservation, **3**, 176-183 **(1994)**.
[6] E.F.George, D.J.M.Puttock, H.J.George; Plant Culture Media, Exegetics Ltd.Westbury, England, **1,2**, **(1987)**.
[7] Y.Kato; Cytologia, **29**,79-85 **(1964)**.
[8] M.D.Materi, L.A.Dionne, B.G.Cumming; Plant Cell Tissue and Organ Culture, **42**, 27-31 **(1995)**.
[9] J.H.Miller, P.M.Miller; Amer.J.Bot., **48**, 154-159 **(1961)**.
[10] E.Sheffield, G.E.Douglas, S.J.Hearne, S.Huxham, J.M.Wynn; Amer.Fern.J., **91**, 179-186 **(2001)**.
[11] M.Thentz, C.Moncousin; Hort.Suis., **57**, 293-297 **(1984)**.
[12] D.P.Whittier; Amer.Fern J., **77**, 95-99 **(1987)**.
[13] D.P.Whittier; Amer.Fern J., **88**, 106-113 **(1998)**.
[14] D.P.Whittier, J.C.Pintaud; Amer.Fern J., **89**, 142-148 **(1999)**.