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FACTORS AFFECTING *IN VITRO* GROWTH AND PROPAGATION OF *FURCRAEA* VAR. GREEN

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SUMMARY

***Furcraea* (*Furcraea gigantea* Vent.) is a beautiful ornamental foliage plant. It is commonly propagated by bulbils only. Conventional method of propagation is rather slow and of longer duration. While standardizing the method of micropropagation of *Furcraea,* the factors influencing *in vitro* growth and propagation of *Furcraea* were examined. Different factors studied were surface sterilants ($HgCl_2$ and sodium hypochlorite); pH (6 levels); sucrose (5 levels) and size of bulbils explants. In case of surface sterilization treatments, better response was noticed with $HgCl_2$ (0.05%) treatment for 5 minutes, which recorded minimum death of culture (12.5 %) with no contamination. Though pH 6.5 recorded cent per cent establishment and maximum shoot growth, reasonably good establishment of the explant (90-100 %) and growth of sprouted shoot was noticed on a pH ranging from 5.5 to 6.5. Root induction was inhibited at pH 5.8. Low sucrose level (1 %) showed maximum establishment with better shoot growth. However, proliferation was better on medium fortified with 2 and 3 % sucrose levels. Among the different size of bulbils, whole bulbils showed higher shoot growth as compared to half bulbils or quarter pieces of bulbils on establishment medium.**

***Key words*: Bulbils, explant, *in vitro*, organogenesis**

INTRODUCTION

Furcraea is a very important ornamental foliage succulent plant. It is grown equally in tropical and sub-tropical conditions even in arid zones. *Furcraea* is propagated by bulbils only. The plant flowers once in life. The flowers are borne on a long stalk and bulbils are borne on this stalk (Anon 1976). These bulbils are used for further propagation (Anon 1956). Thus, conventional method of propagation is rather very slow. It produces 1200-1250 bulbils at the age of approximately 8-10 years. *In vitro* propagation technique has been demonstrated to be suitable large scale plant multiplication (Brans 1979). Micropropagation technique is a rapid method of multiplication and, if standardized, it can meet the demand of elite plants. *In vitro* growth of tissue is affected by different factors, viz. pH of the medium, types of sterilants and their concentration, concentration of sucrose, types of culture vessel and types of explants. No such information is available for *Furcraea* species. Hence, the experiments were planned with a view to examine the influence of the different factors on *in vitro* growth and propagation of *Furcraea* for standardizing the protocol of micropropagation.

MATERIALS AND METHODS

Present investigations on various aspects for standardization of micropropagation of *Furcraea* var. Green were carried out at the Department of Biotechnology, ASPEE College of Horticulture and

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Forestry, Navsari Agricultural University, Navsari. While standardizing the methods for micropropagation of *Furcraea* and the factors influencing *in vitro* growth and propagation of *Furcraea*, different factors were studied, i.e. surface sterilants ($HgCl_2$: 0.05 and 0.1 % each for 3 and 5 min and sodium hypochlorite (NaOCl) 10 % for 5 and 10 min duration dip), pH (6 levels: 5.0, 5.5, 5.8, 6.0, 6.5 and 7.0 pH), sucrose (5 levels: 0.0, 1.0, 2.0, 3.0, and 4.0 %) and size of bulbils explant (F-full; H-half and Q-quarter size).

Bulbils (1.5 to 2.0 cm size) borne on the flower stalk of *Furcrea* var. 'Green' were used as a source of explants. Shoot tips of about 1.5 cm in length were inoculated from bulbils. The explants were washed in running tap water for about 30 min and treated with 10 % solution of detergent for 5 min. The traces of detergent were removed by washing the explants thoroughly with double distilled water. The explants were then surface-sterilized under aseptic conditions in a laminar air-flow cabinet, followed by rinsing them four times with sterile distilled water. The size of sterilized shoot tips was further reduced to 0.8 cm in length by removing the outer massive leaves from the bulbils. The trimmed explants were quickly inoculated on nutrient MS medium (Murashige and Skoog 1962) supplemented with 1 mg/l BAP + 0.1 mg/l NAA + 30 g/1 sucrose and 8 g/1 agar for all factors (Patel and Shah 1999). The cultures were incubated at 26 ± 2^{o} C in culture room and provided with 1000 lux light intensity from fluorescence cool tube lights.

RESULTS AND DISCUSSION

Surface sterilants: The establishment of explants was significantly influenced by sterilant treatments (Table 1). Maximum establishment of explants was recorded in treatments with $HgCl_2$ (0.05%) for 3 min (D_1) and in NaOCl **(**10%) for 10 min (D_6). Although contamination was fully controlled in treatments D_2, D_3, and D_4, death of culture was higher and shoot growth was suppressed in these treatments. Length of shoots was higher at lower concentrations as well as shorter duration of surface sterilizers used. Growth in terms of shoot length was maximum in NaOCl treatments (Fig.1). The sterilization procedure for even the same explant collected from different places may differ owing to difference in microflora and their intensity harboured at different locations. The earlier work of the same laboratory on papaya (Babylatha *et al.* 1997), rose (Patel 1995) and guava (Wali 1996) also supported this view.

Table 1. Establishment, growth and contamination of *Furcraea* explants as influenced by different levels of sterilants. Medium : MS medium, Incubation : 4 weeks

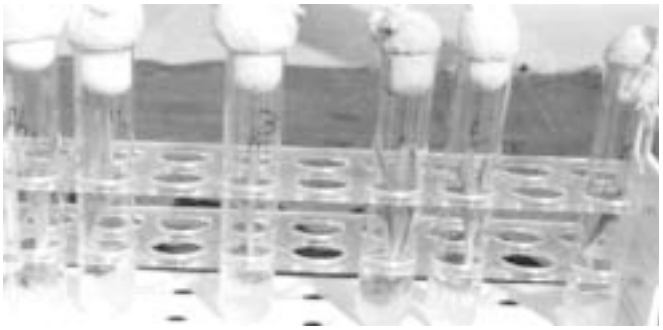
Treatment No.	Establishment response (%)	Contamination (%)	Death of cultures (%)	Shoot length (cm)
D_1 - $HgCl_2$ (0.05 %)-3 minutes dip	87.5(75.61)	12.5	10.0 (contaminated)	1.62
D_2 - $HgCl_2$ (0.05 %)-5 minutes dip	80.5(68.74)	0	12.5	0.94
D_3 - $HgCl_2$ (0.1 %)-3 minutes dip	65(53.91)	0	37.5	1.27
D_4 - $HgCl_2$ (0.1 %)-5 minutes dip	22.5(23.06)	0	77.5	0.68
D_5 - NaOCl (10%)-5 minutes dip	75(64.04)	27.5	27.5 (contaminated)	2.27
D_6 - NaOCl (10%)-10 minutes dip	87.5(74.25)	12.5	12.5 (contaminated)	2.06
S.Em.±	5.21			0.209
C.D. at 5%	14.45			0.592
C.V. %	21.45			14.80

Figure in parentheses are arc sine transformed value.

D_6 D_5 D_4 D_3 D_2 D_1

Fig. 1. Influence of different levels of sterilants on explants of *Furcraea* (Details of treatment given in Table 1)

pH of the medium: From the data given in Table 2, it is clearly shown that establishment of explants and growth was significantly influenced by pH of the medium. Establishment gradually decreased with decrease in the pH from 6.5 to 5.0 and *vise versa*. The maximum establishment and growth was recorded at 6.5 pH (H_5) followed by 6.0, 5.8, 5.5 and 5.0 pH levels (Fig.2). However, growth was at par with 5.5 and 5.0 pH levels. No root induction was noticed at pH 5.8. Green callus was noticed only at pH 6.5. Ambient pH could be decisive for absorption of various nitrogen sources (Street 1966). Growth response curves at different pH indicated that it is nitrate N when pH is acidic (4.7 – 4.9 pH approximately), ammonical N at neutral pH (7.0 – 7.2 pH approx.) and nitrite at pH 5.0 – 6.0 supported maximum growth. The results obtained with *Furcraea* explants may be considered in light of preference of nutrients for establishment and growth. Growth near neutral pH, suggesting favorable effect of ammonical N rather than nitrate N.



H_1 H_2 H_3 H_4 H_5 H_6

Fig. 2. Influence of different levels of pH on explants of *Furcraea* (Details of treatment given in Table 2)

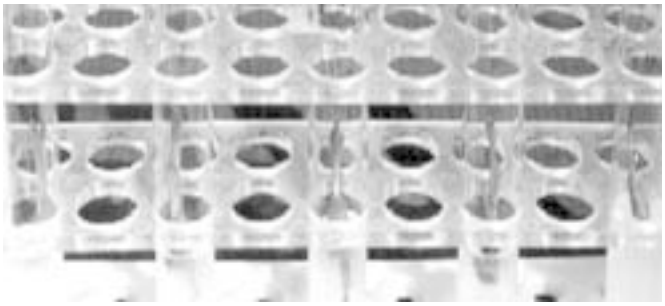
Sucrose concentration in the medium: It is seen from the data given in Table 3 that the establishment was significantly affected by the different concentrations of sucrose (S_1 to S_5) in the medium. Maximum establishment of explants was recorded in 1 % sucrose (S_2) level followed by S_3, S_1, S_4, and S_5 in decreasing order. Callus induction was noticed on the medium containing 1% sucrose (S_2) whereas, sprouting of new shoots was noticed in S_4 and S_3 treatments. Shoot length was highest on medium without sucrose. In general, increasing sucrose concentration decreased shoot length; except at the highest sucrose level tested (4 %; Fig.3). There was an increase in the number of roots and length of roots on medium supplemented with sucrose up to 2 %, beyond which the number of roots decreased to a

Table 2. Establishment and growth of *Furcraea* explants as influenced by different pH levels of the medium. Medium : MS medium, Incubation : 4 weeks

Treatment No.	Establishment response (%)	Shoot length (cm)	No. of roots per shoot	Root length (cm)	Callus status
H_1- 5.0 pH	79.50	3.66	0.83	0.13	-
H_2- 5.5 pH	89.50	3.87	1.60	0.10	-
H_3- 5.8 pH	91.00	3.41	0.00	0.00	Swelling
H_4- 6.0 pH	95.00	2.63	2.16	0.27	Swelling
H5- 6.5 pH	100.00	3.91	0.10	0.10	Green callus
H_6- 7.0 pH	78.57	2.50	3.33	0.23	-
S.Em.±	1.707	0.114			
C.D. at 5 %	4.787	0.319			
C.V. %	8.58	15.31			

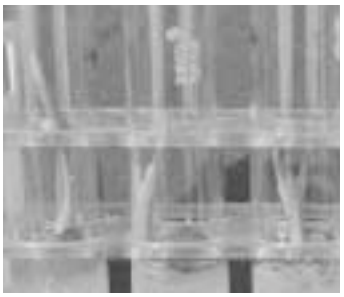
Table 3. Establishment and growth of *Furcraea* explants as influenced by different levels of sucrose of the medium. Medium : MS medium, Incubation : 4 weeks

Treatment No.	Establishment response (%)	Shoot length (cm)	New shoots/ explant	No. of roots/ shoot	Root length (cm)	Callus Status
S_1-Sucrose (0.0 %)	77.5	2.50	0.00	0.53	0.20	-
S_2-Sucrose (1.0 %)	90.5	2.43	0.00	0.71	0.27	Callusing
S_3-Sucrose (2.0 %)	80.0	2.36	0.10	1.27	0.95	-
S_4-Sucrose (3.0 %)	70.5	2.05	0.20	0.90	0.14	-
S_5-Sucrose (4.0 %)	61.0	2.20	0.00	0.50	0.57	-
S. Em. ±	1.798	0.23				
C.D. at 5%	5.057	NS				
C.V. %	10.60	24.60				



S_1 S_2 S_3 S_4 S_5

Fig. 3. Influence of sucrose concentration in the medium on explants growth (Details of treatment given in Table 3)



F **H** **Q**

Fig. 4. Influence of size of bulbils on growth. F-full; H-half; Q-quarter

minimum at the highest level examined. Kumar and Kumar (1998) reported that level of sucrose was maintained between 2 to 3 per cent in majority of the media. However, optimum response in *Furcraea* was obtained at 1.0 per cent sucrose level. The requirement may be related to the specific carbohydrate metabolism through which water relations and endogenous phytohormones are regulated.

Size of bulbil explants- Visual observations revealed that among the different size of bulbil inoculated in the medium, the full size bulbil exhibited maximum shoot growth followed by half size and quarter size of bulbils (Fig. 4). Also, Pierik (1987) stated that *in vitro* plant growth was affect by genotypes, physiogical state and age of tissues or explants. Looking to overall results, it is clear that pH of the medium, sucrose level, size of the explants are the controlling factors *in vitro* growth and overall success of micropropagation of *Furcraea*.

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