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## **EFFECT OF SUCROSE ON *IN VITRO* CALLOGENESIS, EMBRYOGENESIS AND ORGANOGENESIS OF *Dendrobium sabin H.***

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### **ABSTRACT**

Protocorms of *Dendrobium sabin H* were proliferated on Murashige and Skoog (MS) medium supplemented with 0%, 1.5%, 3%, 5%, 7%, 9%, 11% and 13% sucrose to determine the callus growth, embryogenesis and organogenesis. Maximum weight of callus was found to be 365 mg after 20, 40 and 60 days of culture on the medium supplemented with 5% sucrose level. The results showed that there was continuous increase in fresh weight of callus at 20, 40 and 60 days after inoculation. Maximum callus growth was observed when the sucrose concentration was 5%. Lowest and highest levels of sucrose i.e. 0% and 13% showed no growth. The 5% level of sucrose was found to be the most appropriate for the induction of callus timely and efficiently as well. It was also found that 5% level of sucrose took significantly less days (20 days) to form somatic embryoids. Maximum embryogenesis percentage (100%) was observed at 5% followed by 3% (77.77%) sucrose level. No embryogenesis took place at 0.0% and 13% levels of sucrose. The 1.5%, 9% and 11% resulted in a very poor embryogenesis. The 5% level of sucrose produced significantly more number of shoots. Length of the developed shoots was also significantly high at this level than any other level.

**Key Words: Sucrose, *In-vitro* Callogenesis, Embryogenesis, Organogenesis, *Dendrobium sabin H.***

#### **Introduction:**

*In vitro* grown plants are devoid of photosynthesis so they need a readily available source of carbon which is usually provided in the medium at 3%. However optimum level of sucrose varies for different crops and even species. The present experiment was conducted to evaluate the efficacy of different sucrose concentrations; low and high as compared to normal in MS media (George *et al.*, 2008)

Orchids are grown worldwide with almost 8 percent share in world floriculture trade (Asghar *et al.*, 2011). Because of its shape, assorted colour and enduring blooms it is considered to be one of the most outstanding genera in ornamentals (Tokuhara & Mii, 2001). Orchids are of considerable economic importance. Various orchids are used for a variety of folk medicine and cures. (Rafique *et al.*, 2012).

Among orchids, *Dendrobium* occupies a significant position with marvelous varieties like Emma white and many more which are admired for purity of blooms and prolonged shelf life (Vendrame, 2008). *In vitro* propagation of *Dendrobium* is carried out successfully through the use of optimum plant medium and proper growth regulator combinations (Aktar *et al.*, 2008).

Plant tissue culture and micropropagation techniques play a great role in genetic conservation programs and management of botanical collection of orchids in a very efficient manner (Mahendran & Bai 2009). Micro propagation system ensures pathogen-free, true-to-type and rapid production. Selection of suitable media components is very important for improving growth *in vitro* conditions and for this purpose different types of additives are used

(Rafique *et al.*, 2012). The plants produced by exploiting the amazing features of this technology provide greater export potential as they are shipped internationally with limited quarantine restrictions due their quality and it has the great potential for developing new cultivars utilizing artificial nutrient medium under a control sterile environment (Kwa *et al.*, 1995).

The need of the time is establishment of a consistent cloning methodology for orchid which encourages the rapid plant propagation and produces a large number of high quality plants (Asghar *et al.*, 2011). The present project was undertaken to study the effect of different sucrose concentrations on *in vitro* callogenesis, embryogenesis and organogenesis in *Dendrobium* orchids. As a matter of fact it will serve as foundation for mass scale propagation.

#### Materials and Methods:

Studies in *Dendrobium* orchid from pre-developed protocols were conducted to investigate the suitable conditions for *in vitro* callogenesis, embryogenesis and organogenesis in *Dendrobium* orchids. Data was collected on fresh weight of callus (mg) after 20, 40 and 60 days, callus growth, number of days to induce callus, days to embryos formation, embryogenesis %, number of shoots, and length of

shoots (cm). The media were placed in autoclave for 15 minutes at 121°C under 15 psi pressure. The pH of the medium was maintained at 5.8. Following culture conditions were maintained artificially in growth room for *in vitro* regeneration in orchids. Cultures were incubated at 25±2 °C under 16/8 hours photoperiod (2500 lux) with white fluorescent tubes (Philips TL 40W/54). The experiment was laid out according to Completely Randomized Design (CRD) with six replications. Data was analyzed by using statistical software MSTATC. Significance among treatment means was compared using Duncan's Multiple Range (DMR) test (Steel *et al.*, 1997).

#### Results

##### Fresh weight of callus (mg)

The results showed that T<sub>3</sub> resulted in maximum weight of callus after 20, 40 and 60 days of initial culture. Maximum weight of callus was found to be 365 mg after 60 days of culture on the medium supplemented with 5% sucrose level. So 5% level of sucrose was found to be best in all the cases followed by 3% and 7%. 1.5 %. Sucrose concentration at 9% showed significantly low weight in all the observations. The results showed that there was continuous increase in fresh weight of callus at 20, 40, 60 days after inoculation (table 1).

**Table 1: Effect of Sucrose on Fresh Weight of callus**

Treatment	Sucrose concentration (%)	Fresh wt. of callus (mg)		
		20 days	40 days	60 days
T <sub>0</sub>	0.0	No growth	No growth	No growth
T <sub>1</sub>	1.5	52.00 <sup>d</sup>	62.66 <sup>d</sup>	77.111 <sup>d</sup>
T <sub>2</sub>	3.0	106.00 <sup>b</sup>	171.00 <sup>b</sup>	245.89 <sup>b</sup>
T <sub>3</sub>	5.0	220.33 <sup>a</sup>	306.33 <sup>a</sup>	365.44 <sup>a</sup>
T <sub>4</sub>	7.0	103.22 <sup>b</sup>	170.78 <sup>b</sup>	236.56 <sup>b</sup>
T <sub>5</sub>	9.0	73.444 <sup>c</sup>	113.89 <sup>c</sup>	148.44 <sup>c</sup>
T <sub>6</sub>	11	45.556 <sup>d</sup>	55.33 <sup>d</sup>	68.77 <sup>d</sup>
T <sub>7</sub>	13	No growth	No growth	No growth

#### Callus Growth

Availability of optimum food in the medium is necessary for optimum growth. Maximum callus growth was observed obtained in T<sub>3</sub>, the callus growth was observed excellent at 5%, level followed

by 3%, 7% and 9% which resulted in good callus growth, further increased level of sucrose did not result good callus growth and showed a decreasing trend. Lowest and highest levels of sucrose i.e. 0% and 13% showed no growth (table 2). At 0% level

there was no carbon source to support callus growth on the other hand 13% sucrose level rendered the

media hypertonic one thus affecting the availability of nutrients.

**Table 2: Effect of Sucrose on callus growth, number of days to induce callus, days to embryos formation, embryogenesis %, number of shoots and length of shoots**

Treatment	Sucrose conc. (%)	Callus growth	No. of days to induce callus	Days to embryos	Embryogenesis %	No. of shoots	Length of shoots (cm)
T <sub>0</sub>	0.0	-	No callus	No embryos	0.00	0.000 <sup>c</sup>	0.000 <sup>c</sup>
T <sub>1</sub>	1.5	+	25.66 <sup>b</sup>	41.667 <sup>a</sup>	40.66	0.888 <sup>c</sup>	0.133 <sup>bc</sup>
T <sub>2</sub>	3.0	++	22.77 <sup>c</sup>	21.667 <sup>c</sup>	77.77	2.444 <sup>b</sup>	0.422 <sup>b</sup>
T <sub>3</sub>	5.0	+++	20.00 <sup>d</sup>	20.000 <sup>d</sup>	100.00	7.444 <sup>a</sup>	2.688 <sup>a</sup>
T <sub>4</sub>	7.0	++	22.77 <sup>c</sup>	22.778 <sup>c</sup>	66.66	2.000 <sup>b</sup>	0.344 <sup>bc</sup>
T <sub>5</sub>	9.0	++	27.22 <sup>ab</sup>	30.000 <sup>b</sup>	44.44	0.555 <sup>c</sup>	0.111 <sup>bc</sup>
T <sub>6</sub>	11	+	27.22 <sup>ab</sup>	30.000 <sup>b</sup>	22.22	0.000 <sup>c</sup>	0.222 <sup>bc</sup>
T <sub>7</sub>	13	-	No callus	No embryos	0.00	0.000 <sup>c</sup>	0.000 <sup>c</sup>

“-” = no growth, “+” = low growth, “++” = good growth, “+++” = excellent growth

#### No. of days to induce callus

The results show that treatment T<sub>1</sub>, T<sub>5</sub> and T<sub>6</sub> (1.5%, 9% and 11% respectively) took maximum days to induce callus. As shown previously there was no callus growth at 0% and 13 % level of sucrose even after 60 days (all the protocorms died after 20 days of culture). T<sub>3</sub> took significantly less days to induce callus (20 days), followed by T<sub>2</sub> and T<sub>4</sub>. So 5% level of sucrose was found to be the most appropriate for induction of callus timely and efficiently as well (table 2). So, 5 % level of sucrose was found to the best for early induction of callus.

#### Days to Embroids formation

It was found that 5% level of sucrose took significantly less days to form somatic embryos. It took 20 days to form somatic embryos after the proliferation of callus in the same medium (table 2). T<sub>3</sub> was found best followed by T<sub>2</sub> and T<sub>4</sub> taking more time (about 22 days each). T<sub>1</sub> took ignorantly more

days to form embryos followed by T<sub>5</sub> and T<sub>6</sub> (from protocorms as explants). So, 5 % level of sucrose was found to the best for early induction of somatic embryos.

#### Embryogenesis %

Table 2 shows the embryogenesis percentage. It is obvious from the table that there was a considerably high difference of embryogenesis percentage at varying concentrations of sucrose. Maximum embryogenesis percentage (100 %) was observed at 5 % followed by 3% (77.77%) sucrose level. No embryogenesis took place at 0.0% and 13 % levels of sucrose. 1.5 %, 9 % and 11 % resulted in a very poor embryogenesis. The results also indicated that the growth was increased by adding additional sucrose (up to 5%) but not at a very high concentration (table 2) any additional sucrose than 5% level showed negative correlation with the embryogenesis (Fig. 1 and 2).



Figure 1 *In vitro* callogenesis and embryogenesis at 5% sucrose



Figure 2 *In vitro* organogenesis at 5 % level of sucrose

### No. of shoots

The analysis of variance showed that 5% level of sucrose produced significantly higher number of shoots followed by 3% and 7% level of sucrose. The other levels 0%, 11% and 13% (very high levels) produced no shoots while 1.5 % and 9% produced very low number of shoots. So, it is obvious that 5% level sucrose is best for increasing shoot number per explant.

### Length of shoots

Statistical analysis showed that the length of the shoots was found significantly high at 5% level of supplemented sucrose in the media. Shoot length at 1.5%, 3%, 7%, 9% and 11% are non-significant among themselves and in all these shoot length was found to less than 0.5 cm. As no shoots were formed at 0% and 13% so, there is no concept of their length. Maximum shoot length was found to be 2.68 cm at 5% level of sucrose. (Table 2)

### Discussion

#### Fresh weight of callus (mg)

Increased level of sucrose resulted in more callus growth *in vitro* conditions. The present results indicate that the growth was increased by adding additional sucrose due availability of more food in the medium which resulted in more weight of callus. But at a very high concentration this trend does not persist. The reason behind is the lack of readily available food for the explants. Without sucrose there was observed no significant growth rather it was negative with respect to days increase in growth. As there was no carbon source. Mentioned results were found in line with Faria *et al.* (2004) and Yoon *et al.* (2009). Our findings are also supported by Alkhateeb, (2008) reported that 0% and 13% showed negative response and also that tissues grown on medium supplement with 10% of date syrup caused severe reduction in number of somatic embryo possibly due to the osmotic stress.

#### Callus Growth

Availability of optimum food in the medium is necessary for optimum growth. The more growth at 5% level indicates that it is the optimum combination for callus growth in *Dendrobium* orchids. However more concentrated medium discourages growth and no growth at 13%. Similar results were reported by Alkhateeb, (2008). So optimum sucrose concentration in MS medium is very important factor to influence callus growth.

### No. of days to induce callus

Days to induce callus also determine the growth *in vitro* conditions. The optimum availability of sucrose reduces the number of days to induce callus by providing optimum conditions for growth. The present results show that 5 % level of sucrose took minimum days to induce callus as it worked to accelerate growth conditions due to the provision of more sucrose. But much higher concentration or low concentrations of sucrose result in more days to induce callus due to unfavorable growth conditions. The results are in line with Faria *et al.*, (2004) & Yoon *et al.*, (2009). So, a balanced carbon source is necessary in the media. As it is very necessary for good growth *in vitro* conditions.

### Days to Embroids formation

Days to Embroids formation also determine the growth *in vitro* conditions. The optimum availability of sucrose reduces the number of days to Embroids formation by providing optimum conditions for growth. The present results show that 5 % level of sucrose took minimum days to Embroids formation as it worked to accelerate growth conditions due to the provision of more sucrose. But much higher concentration or low concentrations of sucrose result in more days to Embroids formation due to unfavorable growth conditions. The mentioned results are in line with Temjensangba and Deb, (2005), Novotna *et al.* (2007) and Faria *et al.* (2004).

### Embryogenesis %

Alkhateeb, (2008) reported that 0% and 13% showed negative response and also that tissues grown on medium supplement with 10% of date syrup caused severe reduction in number of somatic embryo possibly due to the osmotic stress. The tissues growing at 6% percent showed best growth. Optimum growth conditions necessitate the formation of maximum number of somatic embryoids and embryogenesis%. The present results indicate that 5% level of sucrose resulted in maximum embryogenesis as compared to conventional used level at 3%. Further elevated levels result in less embryogenesis % and the same is true for decreased levels. Mentioned results were found in line with Faria *et al.*, (2004) and Yoon *et al.*, (2009).

### No. of shoot

The mentioned results are also supported by Faria *et al.* (2004) who reported high shoot multiplication (1:4) in the 60 g L<sup>-1</sup> sucrose treatment, even without

the addition of plant hormones. The present results also depict that 5% level of sucrose result in more number of shoots. The reason is obvious, the optimum sucrose level resulted in promotion of shoot number in *Dendrobium* protocorm culture. Similarly higher and lower levels did not provide optimum growth conditions and even retard the growth in extreme cases. The results are in line with Alkhateeb, (2008). They reported 60gm (6 %) significantly affected the *in vitro* growth but 10 % sucrose level reduced the growth (table 2). Similar results were found by Novotna *et al.* (2007) and Temjensangba & Deb (2005).

#### Length of shoots

Current finding is in line with Alkhateeb, (2008) who reported longest shoot at 6% level of sucrose. Our study reveals that length of shoots also varies with the sucrose concentrations. Maximum length of shoots was observed at medium supplemented with 5% sucrose but the reverse trend is observed in other (higher or lower) concentrations. It has reported that the growth rate of *In vitro* cultured plants is a function of net assimilation rate resulting from the uptake of nutrients (Kothari *et al.*, 2004, Hasan *et al.*, 2010) from the media. The mentioned results are also supported by Faria *et al.* (2004) who reported greater increases in plant height and in the 60 g L<sup>-1</sup> sucrose treatment, even without the addition of plant hormones.

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