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Effect of potato extract on *in vitro* seed germination and seedling growth of local *Vanda roxburgii* orchid

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Abstract

An experiment was conducted to investigate the effect of potato extract (PE) on seed germination and seedling growth of *Vanda roxburgii in vitro,* a local orchid. Potato extracts significantly enhanced seed germination and seedling growth. Among the concentrations, PE at 200 mlL^{-1} enhanced per cent germination from 17.2% (control) to 78.24% and was found to be the most suitable one for seed germination. It was also the best among treatments for production of viable protocorms as well as healthy seedlings from seeds. Growth of seedlings in respect of length of shoot, number of root per shoot and length of root per seedling was significantly enhanced in medium supplemented with 100 mlL^{-1} of PE. It may be concluded that PE at optimum concentration could be supplemented in medium for high percentage of seed germination and seedling growth of *Vanda* orchid.

Keywords: Potato extract, Seed germination, Seedling growth, Vanda orchid

Introduction

Vanda (*Vanda roxburgii* R. Br.) produces highly attractive and beautiful cut flowers and pot plants. Most of the orchids are propagated clonally and very few are by seeds. The seeds of orchids are tiny, lack of endosperm and stored food and have rudimentary embryos (Szendrak *et al.,* 2000). Germination of some orchid seeds is slow, cumbersome and relatively inefficient and required a long period to germinate (Sagawa, 1990). Following Knudson's (1922) success of asymbiotic germination, production and commercialization of many orchids have been expanded tremendously. *In vitro* techniques for micropropagation of orchids are well known for their exploitation as a major trade in recent years in developed countries (Sagawa and Kunisaki, 1982). It has been shown that different orchid groups possess distinct nutrient requirements for germination and subsequent development of seedlings (Valmayor *et al.,* 1977). Considering different requirements for seed germination and seedling growth many culture media as well as supplements into medium have been reported by many researchers (Curtis and Spoerl, 1948; Kano, 1965; Islam *et al*., 1999). Hyponex medium is reported as an instant and suitable solution for orchid seed germination (Kano, 1965). Report revealed that etiolated soybean seedling extract and PE supplement in to Hyponex medium have enhanced per cent germination from 38% (control) to 85% in *Cattleya* orchid (Islam *et al*., 2000). Seed germination of *Vanda roxburgii* has not been studied in details relating to the effects of complex organic additives supplement into Hyponex medium. For seed germination and seedling growth *in vitro* some complex organic additives were reported satisfactory while some have shown unsatisfactory and even inhibitory effects (Arditti, 1967; Islam *et al*, 2000). There is no report on seed germination and seedling growth of local *Vanda roxburgii* of Bangladesh. Hence, the present research was planned to investigate the effect of PE on seed germination and seedling growth of *V. roxburgii* with the aim to develop a complete protocol of high frequency seed germination and seedling growth.

Materials and Methods

Mature capsules of *Vanda roxburgii* R.Br collected from Horticulture Research Center, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur, were used as plant materials. The experimentation duration was from June to December, 2010.

A capsule was surface sterilized by immersion in 0.1% $HgCl_2$ aqueous solution for 15 minutes. Then it was washed three times with sterilized distilled water. Inside a flow cabinet the capsule was opened with a sterile scalpel and seeds were suspended in sterilized distilled water and agitated with a glass rod. Then one ml of seed suspension was dispersed uniformly on the medium in each culture flask. This seed suspension contained 1110.4 $\pm$ 102.3 seeds per culture flask. The number of dispersed seeds was determined following the method of Ichihashi (1990).

Two separate cultures were maintained: the first for seed germination and the second one for seedling growth. Hyponex medium (Hyponex Corp. Japan) 3 gL^{-1} supplemented with 20g of sugar and 3 gL^{-1} gelrite (Merck Co. Inc.) was used as basal medium (BM). Potato extract (cv. Lal Pakri) was prepared following Islam *et. al.* (2000) and supplemented into medium. The pH of the medium was adjusted to 5.6 prior to autoclaving for 15 minutes. Each experimental unit consisted of a 10 ml Erlenmeyer flask, containing 30 ml medium. Each treatment was replicated five times. The experiment was designed in CRD and the mean values of the treatments were compared by DMRT (Gomez and Gomez, 1984).

Cultures were maintained at 25±1°C under 16-hour day-length lighting provided by florescent tubes. The light intensity was 45 µ mol m^{-2} s^{-1}, measured by a quantum sensor (Li-COR L1-189, Lincoln, Nebraska, USA) at the level of the culture.

Per cent germination and protocorm and seedling growth were recorded after two months of culture. Germination was defined as the production of one or more protocorms or as shoot formation. Total per cent germination was calculated by considering the number of green shoot and different protocorms. To determine growth of seedling *in vitro*, data on growth parameters were collected after two months of culture in second culture.

Results and Discussion

Seed germination: Seeds turned green in all flasks after 15 days of sowing. After two months, some seeds developed protocorm derived shoots. Some produced multiple yellow protocorms with green shoot primordia. Others developed yellow, white or brown protocorms (Fig. 1). Many seeds remained unchanged. In course of culture, multiple green and yellow protocorms developed shoots but the white and brown seeds did not (Fig. 1). The green and yellow protocorms were viable and important for orchid production. It is reported that the seed germination process of orchid occurs directly through protocorm into plantlet (Lu and Lee, 1999). If the percentage of viable PLB is the highest, then the percentage of plantlet will also be the highest. Potato extract supplemented into medium enhanced seed germination significantly over the control and the highest germination was recorded at 200 ml L^{-1} (78.24%) followed by 100 ml L^{-1} (66.15%, Fig. 2).

Post germination protocorms: After two months of culture, germinating seeds produced different protocorms. Production of protocorms affected significantly by the PE. The production of protocorm with green shoot was the highest (31.20%) at 200 mlL^{-1} followed by 30.6% at 100 mlL^{-1} PE and the lowest (15.18%) was obtained in control (Table 1). Similarly per cent of yellow protocorm with green primordia was 26.0 % at 50 mlL^{-1} PE but 31.2 % at 200 mlL^{-1}. Yellow protocorm per cent was significantly higher with lowest level of PE which did not increase with higher level of supplement. White and brown protocorm per cent were highest in control and gradually decreased with increased level of PE supplement (Table 1) in the medium.

Table 1. Effect of potato extract supplement into BM on protocorm production of orchids

Concentrations (ml/L)	PLB with green shoot (%)	Green round PLB (%)	Yellow PLB (%)	White PLB (%)	Brown PLB (%)
Control	5.69 c	15.18 c	14.62 b	14.29 a	50.22 a
25	21.82 b	26.15 b	25.05 a	4.77 b	22.21 b
50	31.93 a	26.06 b	27.00 a	3.03 bc	11.97 c
100	32.00 a	30.64a	28.60 a	1.95 c	6.81 d
200	33.51 a	31.20a	27.86 a	1.44 c	5.99 d
LSD at 0.05	3.22	2.88	3.03	1.96	3.08

In a column, figures having common letter(s) do not differ significantly at 5% level as per DMRT.

It was observed that seed germination through protocorm production was enhanced by the addition of PE and the effect was concentration dependent (Table 1). PE might support nutritional balance or support metabolic activity needed for higher germination (Arditti and Harrison, 1977). However, present result does not explain which ingredient of PE is responsible and what metabolic activity happened there, and therefore, further study is needed in this respect. Present result agrees with the earlier report of Islam *et al.* (2000) who concluded that organic extracts selectively enhanced seed germination. PE in BM also improved the survival of the protocorms in *Phalaenopsis sp.* (Tsai *et al*., 1993).

Growth of pantlets: Growth in respect of fresh weight per seedling *in vitro* was enhanced by PE in medium compared to that of control (Fig. 3). The highest fresh weight per seedling was produced at 100 mlL^{-1} of PE followed by 200 mlL^{-1} and the lowest value was for control. Length of seedling supported distinctly by PE at all concentrations provided in the present experiment and the best effect was obtained at 100 mlL^{-1} (Table 2). Similarly number of leaf and number of root per seedling were significantly higher at 100 mlL^{-1} compared to that of control and other treatments. All growth parameters of seedlings were the minimum in seedlings cultured under control.

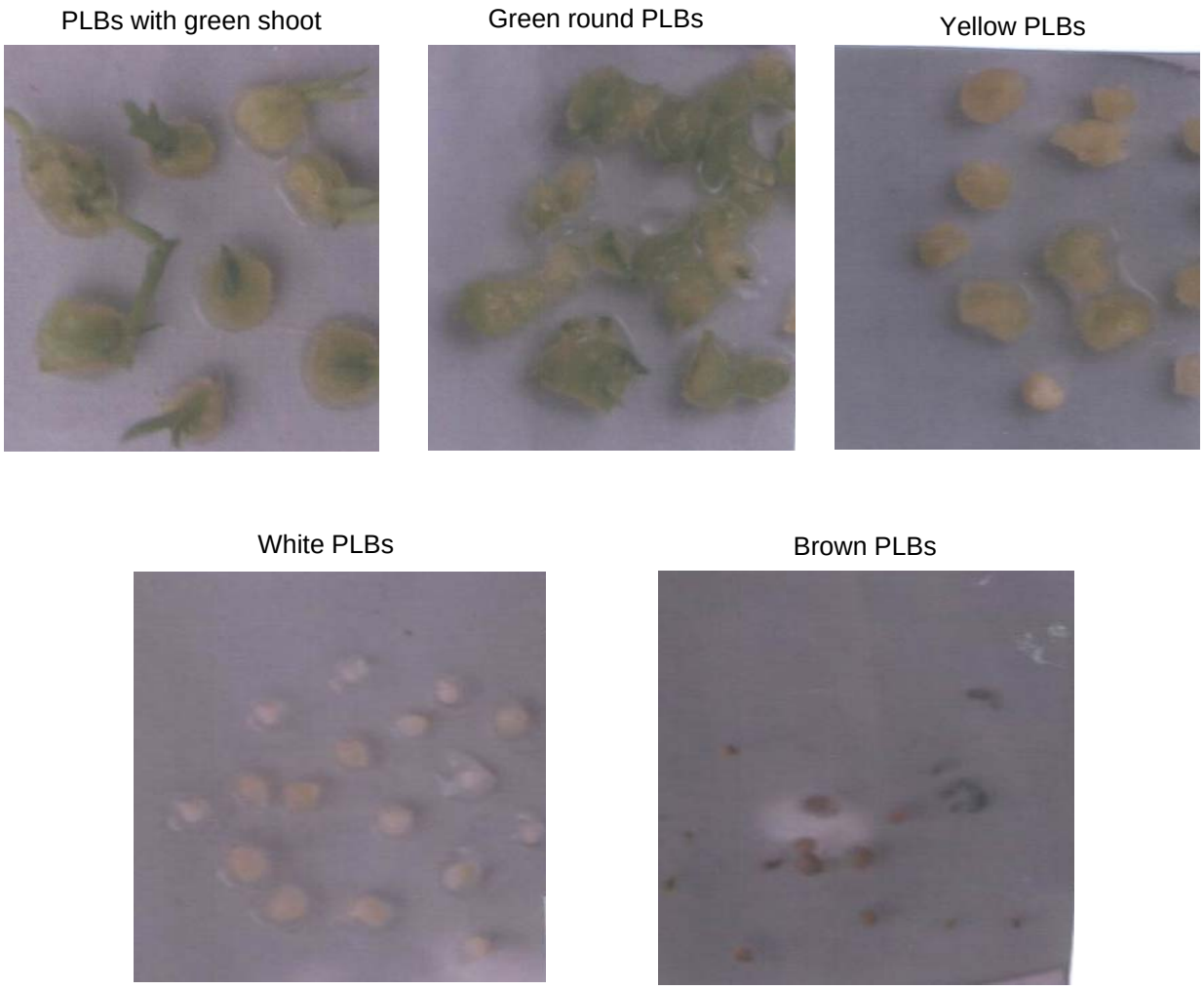

Fig. 1. Germinating PLBs on medium supplemented with different additives

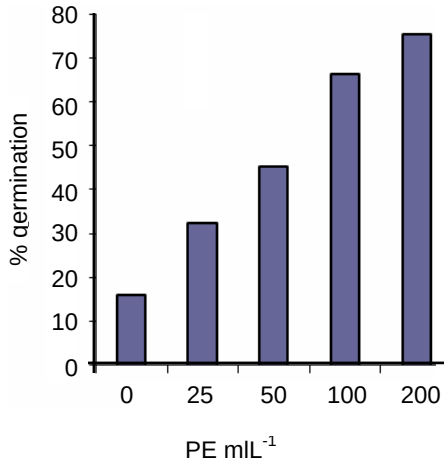

Fig. 2. Effect of PE supplement into medium on seed germination *in vitro*.

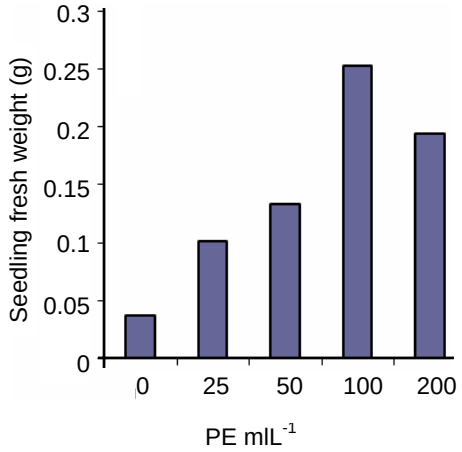

Fig. 3. Effect of PE supplement into medium on seedling growth *in vitro*.

Table 2. Effect of potato extract on growth of orchid seedlings *in vitro*

Concentrations (ml/L)	Length of seedling (cm)	Number of leaves/seedling	Number of roots/seedling	Length of leaf (cm)	Length of root (cm)
Control	1.50 c	2.28 e	2.01 c	1.06 e	0.47 b
25	2.06 b	2.56 d	3.60 b	1.24 d	0.57 b
50	2.29 ab	3.08 c	3.64 b	1.46 c	0.75 a
100	2.35 a	4.32 a	4.64 a	1.89 b	0.76 a
200	2.31 a	3.64 b	3.84 b	2.03 a	0.84 a
LSD at 0.05	0.236	0.398	0.388	0.124	0.152

In a column, figures having common letter(s) do not differ significantly at 5% level as per DMRT.

From the above results, it is evident that PE is effective in seedling growth and the growth parameters were concentration dependent. The requirement of nutrients as well as metabolic activities varies depending on growth stages. There are reports that potato extract contains 1.0 mg of niacin in 100 g raw materials (Anonymous, 1978). These elements might be responsible for growth of orchids. Though most of the growth parameters were the highest at 100 mlL^{-1} of potato extract in *V. roxburgii,* the length of root and length of leaf were the highest at 200 mlL^{-1} of that extract. Above findings indicate that PE is effective in seed germination and concentration as supplement into medium is important for seedling growth *in vitro*. This result agrees with the previous report of Islam *et al.* (2000) in *Cattleya* seedling indicated that potato extract, taro extract and banana homogenate contained niacin and other vitamins. There are other reports that niacin and thiamine can enhance orchid germination and seedling growth (Arditti and Harrison, 1977). Therefore, it is possible that either niacin or thiamine and other components available in potato extract alone or in combination to other organic substances (unknown) enhanced seed germination and seedling growth. This finding also agrees with the previous report of Chen and Chen (1998). They worked on *Oncidium* orchid and reported that plant growth was the best on medium containing extract of banana, carrot, potato, coconut water and tryptone in terms of plant height, leaf number, root number, root length, fresh weight and dry weight. Earlier report also stated that in growth of unorganized tissue (callus) also enhanced by different extracts specially the growth was the best with corn extract (Rahman, 2001). On the other hand, potato and taro with 100 ml/L in medium was the best for callus growth of *Cattleya* orchid. This result agrees with the previous report of Tsai *et al.* (1993). They reported that presence of potato extract in basal medium improved the survival of the protocorms in *Phalaenopsis* orchid. Thus use of potato extract in the medium for germination and seedling growth of selective orchid might enhance orchid production commercially. It may be concluded that PE at 200 mlL^{-1} could be supplemented into medium for higher percentage of seed germination and PE at 100 mlL^{-1} for seedling growth of *Vanda* orchid.

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