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SIMPLE AND EFFICIENT *IN VITRO* METHOD OF STORING *DENDROBIUM* SW. SHAVIN WHITE PROTOCORM LIKE BODIES (PLBs)

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Abstract

In the present study, an efficient and simple *in vitro* method for storing orchid protocorm like bodies (PLBs) has been developed for *Dendrobium* Sw. Shavin. PLBs of *Dendrobium* Shavin White were kept in an empty airtight container without supplementation of any nutrient and stored for up to 135 days under dark condition at different temperatures namely 5, 10, 25 ± 2°C and 30 ± 2°C. The effects of storage period and temperature on the regeneration ability of the stored PLBs were evaluated. The results revealed that PLBs stored for up to135 days at 25 ± 2°C were found to remain unchanged (72%) and recorded higher germination percentage (88) with shoot proliferation ability of 12% after storage. Though, morphologically about 8% of PLBs were found to be brownish green during storage no morphological abnormalities were recorded after 8 weeks of culture. While, storage at 30 ± 2°C only 20% PLBs remained unchanged and 76% of PLBs turned into brownish during the storage which exhibited germination percentage of 16. Though, PLBs showed survival percentage at low temperatures (5 and 10°C), their survival percentages decreased rapidly as the storage duration was increased. The results clearly suggest that PLBs can be stored for only 15 and 75 days at 5 and 10°C respectively. Lower storage temperature of 5°C was not conducive for PLBs because the survival frequency recorded is very low (24) even when stored for a short period (15 days). Thus, PLBs of *Dendrobium* Shavin White can be stored for up to135 days at 25 ± 2°C. Ability to store PLBs is vital as it can ease the distribution of the propagules to the laboratories and commercial nurseries to facilitate the production of uniform plants. Furthermore, this *in vitro* storage method can reduce the need for frequent subculture cycles and conserve the genetic uniformity in germplasm.

Introduction

Orchids are one of the largest flowering plant families among the higher flowering plants with 700 to 800 described genera and 22,000 to 35,000 species (Fadelah *et al.* 2001). Orchids have been popularly grown as an ornamental plant for its exotic beauty and long shelf life of the flowers compared to many other flowers. Among orchids, species of *Dendrobium* Sw. are important commercially in cut flower industry since *Dendrobium* species produce high number of flowers throughout the year and the cut flowers remains fresh devoid of wilting for up to 4 weeks. *Dendrobium* Shavin White is one of the popular hybrids of *Dendrobium* Queen Florist and *Dendrobium* Walter Oumae orchids. Its popularity is mainly because of its increased durability and produces many flowers all year around (Xiang *et al.* 2003, Bustam *et al.* 2013). The orchid industry shows a high potential with the international business covering about 8% of the total world floriculture trade (Chugh *et al.* 2009). This industry plays an important role in Malaysian economy as Malaysia is currently ranked third in the world ranking as orchid-producing country behind Thailand and Singapore. In Malaysia, the export potential of orchids earns about US$ 13 million per annum (Bustam *et al.* 2013). Today, micropropagation is preferred for commercial propagation of all types of orchids as they have the advantage of producing true-to-type as well as

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virus-free plants. Micropropagation through initiation of PLBs is considered as an efficient method because it can proliferate rapidly to produce large number of PLBs in a short period (Sheelavanthmath *et al.* 2005). PLBs are unique form of somatic embryo (SE) which has the capacity to mimic zygotic embryo in natural seed with respect to regeneration to form a complete plantlet. However, SE differs from zygotic embryos as they grow continuously without having dormancy period. After somatic embryogenesis, the embryo will directly convert into plantlet right after it reaches the maturity level (Sterk and de Vries 1993). At the same time, it also has the capability to induce new secondary SEs. This result in heterogeneous growth in one batch of culture where, some are still at the early stage of development while others are already forming shoot (Bustam *et al.* 2013). Thus, it is difficult to provide high number of uniform plantlets at a time and this could be a major problem in the production of orchid for commercial purposes. Due to the heterogeneous growth, it also requires frequent subculture which further adds to the media and labor cost. In addition, the increase in frequency of subculture can induce somaclonal variation and reduce the quality of the PLBs (Minoo *et al.* 2006). Hence, the growth of SEs should be arrested until the required number of embryos is achieved. This can be done through storage method where the development of SEs to form into plant is arrested and the process can be reinitiated again when the favorable environment for conversion is provided (Florin *et al.* 1993). Thus, storage of SEs can permit the production and accumulation of thousands of plant materials for planting to the field (Fujii *et al.* 1993). In addition, the ability to arrest and store SEs i.e. *in vitro* propagules not only reduces further growth but also helps to reduce the sub culture frequency and thus lower the labor cost (Ozudogru *et al.* 2011).

Induction of a reversible quiescent phase in PLBs as that occurs in natural seed will be essential especially for storage purposes (Gray 1989). Previous study has been conducted on the induction of slow growth of *in vitro* explants by the addition of mannitol (10 - 15 g/l) and lowering the sucrose levels (15 - 10 g/l) in the culture medium (Minoo *et al.* 2006). *In vitro* storage reduces the metabolic rate so that further development in PLBs can be slowed down. Temperature has been advocated to play an important role in the induction of quiescent state in PLBs. Storage of PLBs can be achieved by placing in an air tight closed container at a specific temperature which depends on species and hence, need to be standardized for each plant species. Previous study has attempted to store PLBs at low temperature as this condition often reduces the metabolic rate thus prolonging the shelf life (Datta *et al.* 1999, Saiprasad and Polisetty 2003, Mohanraj *et al.* 2009, Sarmah *et al.* 2010). However, this is not a universal phenomenon, as Kishi and Takagi (1997) reported that PLBs can be successful stored at 25°C. In addition, studies also have been developed to induce quiescent phase in SEs by placing SEs in sterile empty culture dishes at controlled relative humidity which is at 70% (Gray 1989). However, both studies mentioned above have their own limitations as per Danso and Ford-Lloyd (2003), the exchange of *in vitro* plantlets in the glass container is problematic. The glass vessels are fragile and often liable to break during transit. Therefore, developing a method of storing PLBs using simple low cost containers is of utmost importance. In this regard, use of small plastic tubes or containers can be a better option as they are cost effective. Thus, this study was undertaken to develop a simple storing method of PLBs in the viable form without using the glass containers which may ease the delivery processes. In this experiment, the PLBs were kept in a sterilized screw capped air-tight polypropylene cryovials (measuring 3.5 × 1.0 cm). This technique is also practical in terms of space and cost as compared to the general slow growth *in vitro* storage. Also, we evaluated the influence of storage temperatures (5, 10, 25 and 30°C) on shelf life and survival of *Dendrobium* Shavin White PLBs stored for longer duration (up to135 days).

Materials and Methods

Plant materials and PLBs multiplication: The starting materials for the experiments were protocorm-like bodies of *Dendrobium* Shavin White obtained from Serdang Orchid Nursery, Selangor, Malaysia. The PLBs were initially induced from culture of *Dendrobium* Shavin White shoot tip *in vitro*. These PLBs were used as a starting material (Fig. 1a) to proliferate new secondary PLBs to obtain the desired number for the entire experiment. PLBs were multiplied on half strength MS liquid medium supplemented with myo-inositol (50.0 mg/l), glycine (1.0 mg/l), nicotinic acid (0.25 mg/l), pyridoxine HCL (0.25 mg/l), thiamine HCL (0.05 mg/l), ferrous sulfate (13.9 mg/l), Na_2 EDTA (18.65 mg/l), sucrose (15 g/l) and BAP (1.0 mg/l) and were placed on a rotary shaker at 90 rpm. The pH of the medium was adjusted between 5.7 and 5.8 with 0.1 M NaOH and 0.1 M HCl before autoclaved at 121°C and 15 psi for 15 min. The cultures were maintained at 25 ± 2°C and 60% relative humidity under an 8 hrs dark/16 hrs light photoperiod using 60 µmole/$m^{2/}$s irradiance (standard cool daylight fluorescent light). Subculture was carried out at every 4 weeks interval. All the culture procedures were done under a sterile condition inside the laminar air flow cabinet.

Storage of PLBs: Vigorous PLBs measuring 3 - 5 mm with small shoot were identified (Bustam *et al.* 2013) and isolated aseptically from PLBs clumps served as the experimental materials. The single isolated PLBs were cultured for one week on half strength MS liquid medium devoid of plant growth regulator (Fig. 1b). After one week, the PLBs were taken out from the culture flask and washed with sterilized distilled water and blotted dry on a sterilized filter paper. The PLBs surfaces were air dried in the laminar air flow cabinet for 30 min. Later, PLBs were placed in a sterilized screw capped air-tight polypropylene cryovials (1.8 ml; Nunc, Denmark) measuring 3.5 × 1.0 cm (Fig. 1c). Five PLBs were inserted in a single container and were repeated five times for every storage temperature. The containers were kept in darkness at commercial refrigerator (5°C), incubator (10°C), air conditioned room (25 ± 2°C) and normal room condition (30 ± 2°C).

Morphological categorization of post-stored PLBs: The post-stored PLBs were taken out after every 15, 45, 75, 105 and 135 days to observe the morphological changes. The morphological categorizations of post-stored PLBs were determined at all defined durations from each storage temperature. The post-stored PLBs were categorized into five different categories namely; unchanged, germinated, brownish green, brown and light green.

Plantlet regeneration from post-stored PLBs: After every defined storage period, the post-stored PLBs were cultured onto the re-growth medium which was half strength MS semi-solid devoid of plant growth regulator and solidified with 3.7 g/l gelrite (Duchefa Biochemie, Haarlem, Netherlands). The PLBs were cultured for 8 weeks to determine the germination and conversion percentage. The germination percentage was recorded after 4 weeks of culture. The PLBs were considered germinated when they produced shoot measuring more than 3 mm. The term conversion was used when the PLBs formed plantlet having leaves and roots (embryo-to-plant development). Detailed observation on the surviving PLBs was carried out for samples recovered from each of the storage. This was done by morphologically categorizing the cultured PLBs after 8 weeks. The post-cultured PLBs were categorized into four namely; normal plantlet having healthy leaves and roots (Fig. 1d, i), proliferation of small multiple shoots or secondary PLBs (Fig. 1d, ii), browning and whitening.

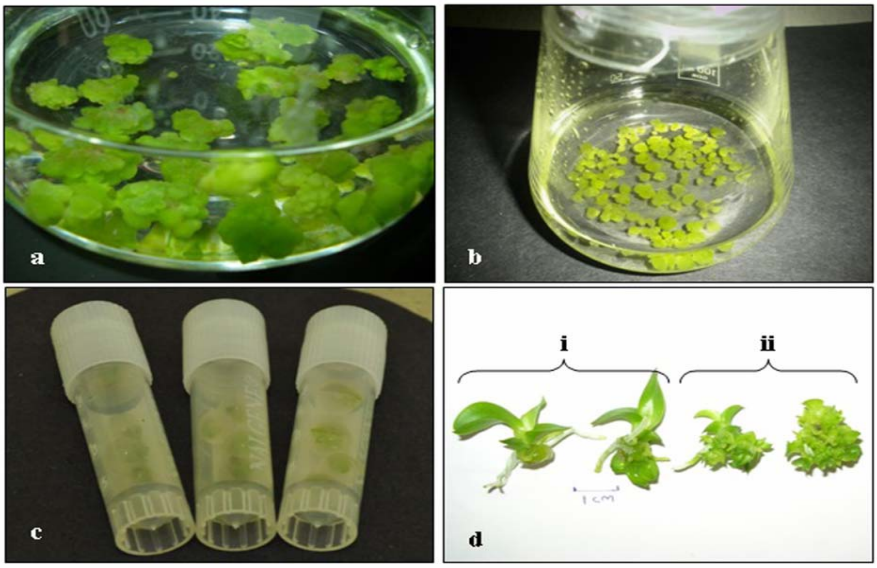

Fig. 1. Clumps of PLBs that were used as a starting material (a), the single isolated PLBs cultured for one week on half strength MS liquid medium devoid of plant growth regulator (b), PLBs in a sterilized cryovials (c) and regenerating PLBs after storage (d); normal plantlet having healthy leaves and roots (i) and proliferation of small multiple shoots or secondary PLBs (ii).

Experimental design and statistical analysis: All experiments were carried out with five replications with five samples for every treatment. Data from all experiments were ANOVA using Statistical Analyses System Software (SAS) release 9.0. This study was laid out in CRD. Means for data were differentiated using DMRT at $p \leq 0.05$ level of significance.

Results and Discussion

The results of morphological changes observed in PLBs after storage for up to 135 days at varied storage temperatures (5, 10, 25 and 30°C) are presented in the Table 1. There was a high percentage of PLBs remaining unchanged morphologically after being stored for up to 135 days at 25°C compared to the other storage temperatures (5 and 10°C). This percentage was found to decrease from 100 to 72% when the duration of storage was increased. Apart from being remaining unchanged, some of the PLBs germinated or turned into brownish green. However, the value is considered low with only 8 - 20% of PLBs germinating after being stored for more than 15 days, while 4 - 8% of PLBs turned into brownish green after being stored for more than 45 days. Storage at 30°C for up to75 days were also found to be considerably having high percentage of unchanged PLBs (88 - 100%).

However, this value was drastically reduced to 20 - 40% when stored for more than 75 days and some of the PLBs germinated and turned into brown color. Most of the PLBs stored at 10°C for more than 15 days turned brown or light green. Whereas, storage at 5°C showed all PLBs turning to light green as the duration of storage increased to more than 45 days. Figure 2 clearly shows the variation in morphology of post-stored PLBs at all different storage temperature after 135 days. PLBs stored for up to135 days at 25°C showed 72% of unchanged PLBs while, increased temperature (30°C) effected significantly on the morphology of PLBs with only 20% of them remained unchanged. Whereas about 76% of the PLBs stored at 30°C turned into brown.

Most of PLBs stored at 10°C turned into light green (60%) and all PLBs stored at 5°C turned into light green. Morphological observations clearly indicate that PLBs of *Dendrobium* Shavin White can be stored for up to15 days at low temperature of 10°C.

Table 1. Influence of different storage temperatures on morphology of post-storage PLBs.

Storage time (day)	Storage temperature (ºC)	PLBs morphology category after storage (%)				
		Unchanged	Germinated	Brownish green	Brown	Light green
15	05	32 ± 10.96c	0 ± 0a	0 ± 0b	0 ± 0a	68 ± 10a
	10	84 ± 8.94b	0 ± 0a	16 ± 8.94a	0 ± 0a	0 ± 0b
	25	100 ± 0a	0± 0a	0 ± 0b	0 ± 0a	0 ± 0b
	30	100 ± 0a	0 ± 0a	0 ± 0b	0 ± 0a	0 ± 0b
45	05	0 ± 0c	0 ± 0a	0 ± 0b	0 ± 0a	100 ± 0a
	10	32 ± 17.89b	0 ± 0a	32 ± 17.89a	0 ± 0a	36 ± 21.90b
	25	92 ± 10.94a	8 ± 10.94a	0 ± 0b	0 ± 0a	0 ± 0c
	30	96 ± 8.94a	0 ± 0a	4 ± 8.94b	0 ± 0a	0 ± 0c
75	05	0 ± 0c	0 ± 0c	0 ± 0b	0 ± 0b	100 ± 0a
	10	0 ± 0c	0 ± 0c	16 ± 0a	48 ± 17.89a	36 ± 10.95b
	25	80 ± 0b	12 ± 10.95a	8 ± 10.95ab	0 ± 0b	0 ± 0b
	30	88 ± 10.95a	4 ± 8.94ab	0 ± 0b	8 ± 10.95b	0 ± 0b
105	05	0 ± 0c	0 ± 0b	0 ± 0a	0 ± 0c	100 ± 0a
	10	0 ± 0c	0 ± 0b	0 ±0a	60 ±14.14a	40 ± 14.14b
	25	76 ± 16.73a	20 ± 14.14a	4 ± 8.94a	0 ± 0c	0 ± 0c
	30	40 ± 20b	12 ± 10.95ab	0 ± 0a	48 ± 10.95b	0 ± 0c
135	05	0 ± 0c	0 ± 0b	0 ± 0a	0 ± 0c	100 ± 0a
	10	0 ± 0c	0 ± 0b	0 ± 0a	40 ± 14.14b	60 ± 14.14b
	25	72 ± 10.95a	20 ± 14.14a	8 ± 10.95a	0 ± 0c	0 ± 0c
	30	20 ± 0b	4 ± 8.94b	0 ± 0a	76 ± 8.94a	0 ± 0c

Mean ± Sd with different letters within column for each of the storage durations are significantly different based on DMRT ($p \leq 0.05$).

In contrast, higher temperatures of 25 and 30°C were found to be more suitable for storing PLBs. At 25°C PLBs can be stored for up to 135 days while at 30°C PLBs lasted for 75 days with no morphological changes observed on the post-stored PLBs. Further, these observations on PLB morphology was confirmed by the regeneration test by culturing post-stored PLBs on half strength MS semi-solid medium devoid of plant growth regulators. Thus, morphology of post-stored PLBs can be used as an indicator to determine the survival potential. Based on the germination test after storage, it was noticed that PLBs survived longer when stored at 25°C compared to PLBs stored at 5, 10 and 30°C (Fig. 3). Though PLBs stored at 25°C maintained high viability for up to 75 days, there was a slight reduction in the germination percentage as the storage was increased. About 96 and 88% of germination percentage was recorded after 105 and 135 days of storage respectively. Yet, storage at 25°C was found to show higher regeneration compared to any other storage temperatures tested for the same duration of storage. PLBs stored at 30°C had high germination percentage which was between 96 and 100% when stored for up to 75 days and it was comparable to the storage temperature of 25°C with no significant statistical difference. However, drastic decline was recorded on the germination percentage of PLBs stored for 105 and 135 days which

were 52 and 24%, respectively. High germination percentage (96) was obtained when PLBs were stored at 10°C for 15 days while, further incubation period of 45 and 75 days showed decline of germination capacity with 24 and 16%, respectively. Interestingly, there was no germination observed after 75 days of storage. Storage at 5°C had the lowest germination percentage when stored for 15 days and there was no germination observed after 45 days of storage. In the present study, the post-stored PLBs were capable to regenerate into complete plant on re-growth medium (Table 2). It is evident from the results that the conversion frequency of PLBs into plantlet was not affected even when being stored for up to135 days at 25°C. Storage at 25 ± 2°C could retain high conversion capability where 88 - 100% of stored PLBs turned into plantlet after 8 weeks of culture even after being stored for 135 days (Fig. 4). High conversion percentage (92 - 100) could be obtained from PLBs stored at 30 ± 2°C for up to 75 days only. The conversion percentage declined drastically after 75 days of storage (16 - 48). While for storage at 10°C, high conversion percentage (92) could be achieved only when PLBs were stored for 15 days.

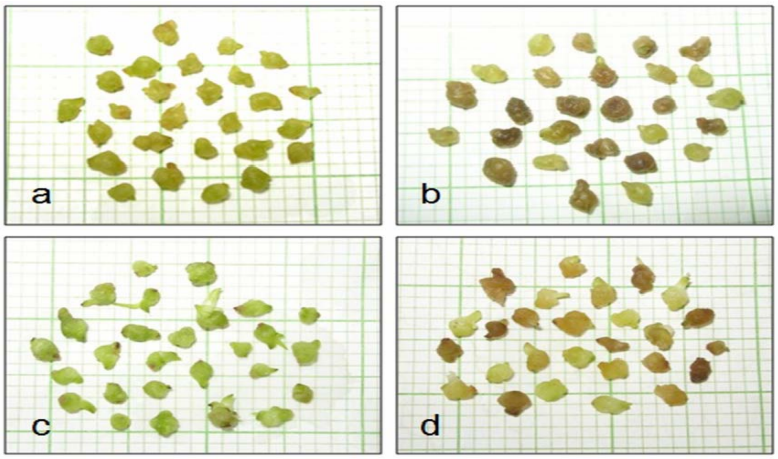

Fig. 2. Morphological categorization of PLBs after 135 days of storage at (a) 5°C, (b) 10°C, (c) 25 ± 2°C and (d) 30 ± 2°C.

The conversion percentage declined drastically when stored for 45 and 75 days (20 and 12 respectively) and no conversion was recorded after storage for more than 75 days. Conversion after storage at 5°C could be obtained only when PLBs were stored for 15 days (24%) and the percentage was low as compared to the other storage temperatures at the same duration of storage. Apart from having the capability of converting into plantlet after culture, some of the survived PLBs could regenerate to form small multiple shoots or proliferated to form new secondary PLBs. Although the percentage of PLBs forming into plantlet after 15 days of storage at 5°C was low, 60% of them were survived because they could regenerate into small multiple shoots or proliferate to form new secondary PLBs (Table 2). PLBs which did not survive after storage were unable to regenerate and their morphological examinations showed browning or whitening depending on the temperature of storage. Storage at 10 and 30°C resulted in browning of PLBs after storage period of more than 15 (24 - 60%) and 75 days (48 - 76%). All PLBs stored at 5 and 10ºC for more than 15 days turned into brown or white when cultured on re-growth media and failed to germinate.

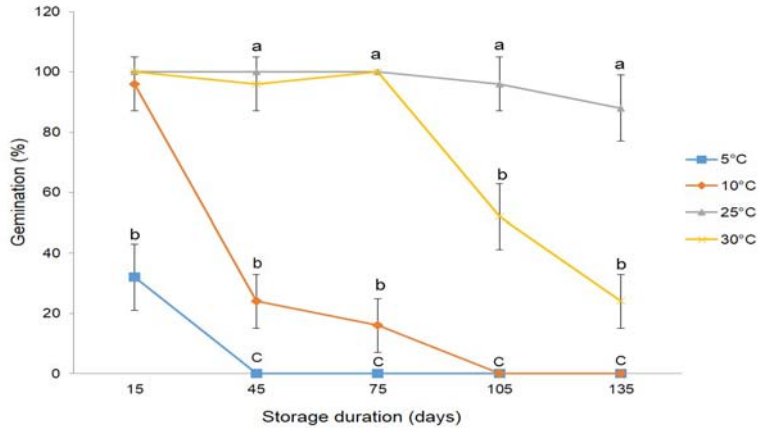

Fig. 3. Influence of different storage temperatures on germination percentage of PLBs. Means with different letters within column are significantly different based on DMRT ($p \leq 0.05$).

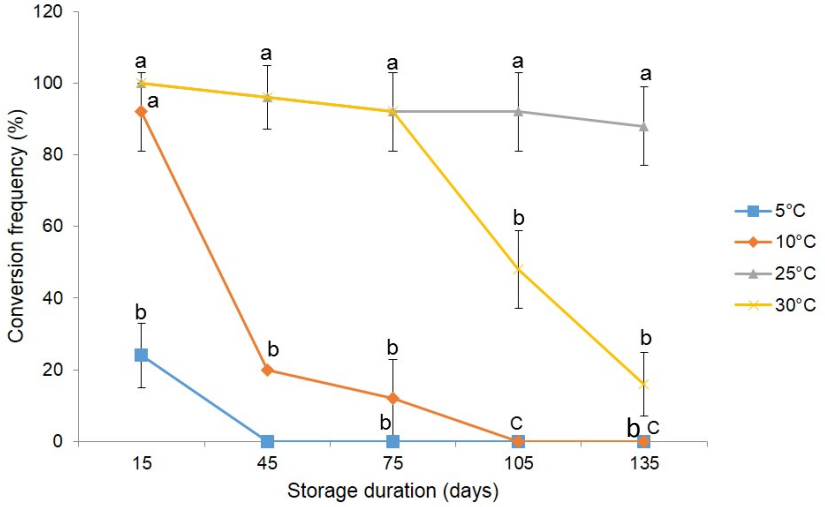

Fig. 4. Influence of different storage temperatures on conversion percentage of PLBs. Means with different letters within column are significantly different based on DMRT ($p \leq 0.05$).

Present findings have revealed an efficient method for storing *Dendrobium* Shavin White orchid PLBs for up to135 days. A simple storage method was developed in this study by keeping PLBs in darkness in an empty small air tight container without supplementation of nutrient media or application of growth retardant to induce dormancy during *in vitro* conservation. In this condition, the quiescent phase in PLBs was induced and this could minimize the demand and risk of regular subculturing which may induce the chance of contamination and somaclonal variation. According to Gupta and Mandal (2003), application of growth retardant and growth limitation condition for *in vitro* conservation may create abnormality to the plant growth which later it will develop into the physiologically stunted or abnormal plant after storage. In addition, our current storage method has met the principal of slow growth as stated by Sharma *et al.* (2003) where

PLBs growth and development can be slowed down by reducing light supply and the use of minimal growth media as this condition causes no bad impact to the system. Nyende *et al.* (2003) reported that storage of naked propagules was not successful and turned brown due to desiccation and lack of external nutrients necessary for growth, and did not regenerate when transferred onto medium. In contrast, our findings revealed a successful storage system for naked PLBs of *Dendrobium* Shavin White for relatively a long period. Also, this is the first report to prove that storing of naked PLBs is better as compared to the encapsulated method explained in our previous

Table 2. Influence of storage temperatures on morphological categorization of PLBs upon culture after storage.

Storage time (day)	Storage temperature (ºC)	Morphological categorization after 8 weeks (%)			
		Plantlet (single/dominant)	Shoot proliferation of PLBs	Browning	Whitening
15	05	24 ± 8.94b	60 ± 14.14a	12 ± 10.95a	4 ± 8.94a
	10	92 ± 10.95a	8 ± 10.95b	0 ± 0b	0 ± 0a
	25	100 ± 0a	0 ± 0b	0 ± 0b	0 ± 0a
	30	100 ± 0a	0 ± 0b	0 ± 0b	0 ± 0a
45	05	0 ± 0c	0 ± 0b	0 ± 0b	100 ± 0a
	10	20 ± 14.14b	20 ± 14.14a	24 ± 21.91a	36 ± 21.91b
	25	96 ± 8.94a	4 ± 8.94b	0 ± 0b	0 ± 0c
	30	96 ± 8.94a	4 ± 8.94b	0 ± 0b	0 ± 0c
75	05	0 ± 0b	0 ±0a	0 ± 0b	100 ± 0a
	10	12 ± 10.95b	8 ± 10.95a	52 ± 10.95a	28 ± 10.95b
	25	92 ± 10.95a	8 ± 10.95a	0 ±0b	0 ± 0c
	30	92 ± 10.95a	0 ± 0a	8 ± 10.95b	0 ± 0c
105	05	0 ± 0c	0 ± 0a	0 ± 0c	100 ± 0a
	10	0 ± 0c	0 ± 0a	60 ± 14.14a	40 ± 14.14b
	25	92 ± 10.95a	8 ± 10.95a	0 ± 0c	0 ± 0c
	30	48 ± 10.95b	4 ± 8.94a	48 ± 10.95b	0 ± 0c
135	05	0 ± c	0 ± 0b	0 ± 0c	100 ± 0a
	10	0 ± 0c	0 ± 0b	40 ± 14.14b	60 ± 14.14b
	25	88 ± 10.95a	12 ± 10.95a	0 ± 0c	0 ± 0c
	30	16 ± 8.94b	8 ± 10.95ab	76 ± 8.94a	0 ± 0c

Mean ± Sd with different letters within column for each of the storage durations are significantly different based on DMRT ($p \leq 0.05$).

report where only 52% of encapsulated PLBs survived after 135 days of storage at 25 ± 2°C (Bustam *et al.* 2013) in the same orchid. This might be correlated to the use of small air tight container without any nutrient supply retains the PLBs in a quiescent state with low metabolic rate for extended period. Likewise, Richard *et al.* (1991), sealing can make culture vessels less permeable to exogenous atmosphere and embryogenic tissue in the culture vessels can be stored for long period without loss of viability or regeneration competence. Thus, a minimal gaseous exchange in the culture vessels will allow SEs to remain in a quiescent phase. In general, storage temperatures play a significant role in the growth of plants and likely regulate the *in vitro* morphogenetic response of PLBs (Omar *et al.* 2012). Likewise, in present study also there was

a varied response of stored PLBs at different temperature. The results clearly suggested that temperature of 25°C as the most suitable to be used for storing naked PLBs as compared to the lower or higher temperature (4, 10 and 30°C). Likewise, PLBs of *Darwinara* and *Brassocattleya* were successfully stored at 25°C but could only able to store for a shorter duration of 84 days with 70% of post-storage survival rate (Kishi and Takagi 1997). In comparison to their results, our study showed better survival rate (88%) of *Dendrobium* Shavin White PLBs even after 135 days. In the same way, Gantait *et al.* (2012) have reported the highest conversion (70.2 %) and germination frequency (76.9%) of stored alginate-encapsulated PLBs of *Aranda* Wan Chark Kuan 'Blue' × *Vanda coerulea* Grifft. ex. Lindl. (AV), a hybrid orchid up to 180 days at 25°C. Storage at 30°C was effective only for up to 75 days with a rapid decline in the survival percentage for longer storage. This could be attributed to the fact that the high temperature induces increased metabolic rate causing rapid deterioration of stored PLBs. Storage at 25°C was found to be better for practical purposes than at lower temperature such as 4°C, because no special facility like refrigeration was required and the application of cold stress will be avoided (Gupta and Mandal 2003). In addition, the equipment and maintenance of low temperature for *in vitro* conservation is costly especially in the country with the tropical climate. Therefore, in many genebanks, slow growth cultures are stored at around 25°C for conservation. At this temperature, there are about 100 species were successfully maintained *in vitro* at NBPGR with 8 - 24 months subculture interval. The results clearly showed that PLBs of *Dendrobium* Shavin White cannot be stored at low temperature of 5°C or 10°C. Similar results were also observed in *Geodorum densiflorum* (Lam.) Schltr (Datta *et al.* 1999) and *Darwinara* and *Brassocattleya* (Kishi and Takagi 1997) where no viability or regeneration was found after storage at 4°C. According to Luo *et al.* (2009), incubation at low temperature of 5°C inhibits the conversion of PLBs to shoots. Furthermore, incubation of PLBs at 10°C for short period resulted in high conversion but drastically reduced when the period extended for more than 14 days. This could be due to the nature of PLBs with higher moisture content. Plant tissues that contained high moisture did not tolerate low temperature which could be the causal factor for the cell death due to chilling injury (Kishi and Takagi 1997). The storage behavior of PLB seems to be like that of recalcitrant seeds which contain high moisture content, thus cannot tolerate desiccation and storage at low temperature (Florin *et al.* 1993).

In conclusion, storage of *Dendrobium* Shavin White PLBs for up to135 days with no or small reduction in survival percentage can be achieved by storing PLBs in a small empty air tight container at 25°C in dark condition. This is an efficient, very simple, cost effective approach and requires no experienced technical workers and advanced equipment for storage. Further, this study offers a strong basis for establishing and optimizing reliable *in vitro* storage protocols for other orchid species in near future.

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