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Variation in Response of Seeds of Three Tropical Orchid Species Towards Storage Conditions for Seed Viability

NOREN S.K.[1] AND ASIT KUMAR BASU[2]

[1]School of Crop Improvement, College of Post-Graduate Studies,
Central Agricultural University (Imphal), Umiam 793103, Meghalaya
norensingh27@gmail.com
[2]Department of Seed Science and Technology,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia 741252, West Bengal
asitkumarbasu@gmail.com

ABSTRACT: Ripe seeds collected from undehisced and almost matured capsules of three tropical Orchid species *viz*., *Phaius tankervilliae*, *Rhyncostylis retusa* and *Aerides odoratum* were first used for standardization of concentration of Tetrazolium (Tz) solution and minimum time required for its viability testing. Another part of the collected capsules/seeds were dried in ambient room temperature for one month wrapping with butter paper. Seeds scooped from such dried capsules were wrapped within a piece of butter paper separately for each species and stored in oven dried test tubes prior set with a little amount of $CaCl_2$ (1:50:: desiccant: seed) covered with a small amount of cotton at the bottom of the test tubes. Test tubes with seeds were plugged with cotton and closed with parafilm, then stored in three different conditions *viz*., in fridge at 4°C, in culture room (25±2°C) and in room temperature. Stored seeds were evaluated for its viability at three intervals- 90, 180 and 270 days of storage (DOS). Viability testing using Tz solution was suitable for Orchid species under study with specific duration as: 24 hours treatment at 0.5% solution for *P. tankervilliae*, whereas it was only 3 and 2 hours at 0.5% solution for *R. retusa* and *A. odoratum* respectively. Out of three storage conditions, fridge storage was found to be better for two species- *P. tankervilliae* and *R. retusa*, whereas the result could not be ascertained in case of *A. odoratum*. Therefore, variation in potentiality of retaining seed viability under different conditions was mainly depended on the genetic architecture of individual species as well as its interaction with storage conditions.

Key words: Orchid, Tetrazolium test, Seed viability, Seed storage

Orchids are popular among the ornamentals due to diversity and beauty of its long lasting and colorful flowers. Being among the most beautiful flowers, orchid occupies an important position in nature with its bewildering range of flower size, shape and colour. It accounts for 3% (approx.) of global cut flower production in terms of value. Orchid dealers mainly depend on collection of plants from its natural habitat (forests) to meet a major part of their demand, both local and foreign. Due to such ruthless collection, rare species are presently confronting the inevitable danger of depletion.

To preserve such rare and threatened orchid species, development of efficient and practical propagation method is the demand of the present day. Conventionally, orchids are propagated by vegetative means, which are time consuming and large scale propagation is practically impossible. Suitable propagation method is *in vitro* seed germination [1-3]. The restricted development of practical propagation methods for orchids is mainly due to lack of viable seeds. In nature, orchid seed can germinate only 0.2-0.3% only which may differs among different orchids again. Sowing seeds on a suitable culture medium and counting germination thereafter is the only direct method to measure seed viability. This is a lengthy and time consuming test and it may take several weeks or months. Therefore, commercial growers require a test procedure, which is fast and easy to perform. This study has two main objectives. They are:

1. To study the response of three orchid species, *viz*. *Phaius tankervillae, Rhyncostylis retusa and Aerides odoratum* to the concentration and duration of treatment in Tz solution for seed viability testing.

2. To study the response of these orchid species to storage condition and duration in respect to seed viability

MATERIALS AND METHODS

As it is done for other crop plants, Tetrazolium test might be a possible method, as developed by Lakon [4] where assessment of seed viability can be made within 24 hours. Ripe seeds from collected capsules were utilized for standardization of both concentration of TZ solution and minimum time requirement for individual orchid species. The concentrations used were 0.1%, 0.5% and 1%. The seeds were soaked in distilled water and kept on a rotatory shaker at 80-90 RPM for 24 hours. A small amount of filtered seeds was placed in small homeopathic vial for each treatment with 10 ml Tz solution. The vials were then tightly closed with cork and kept in dark at 30±2°C. Shaking of those vials at 10 min interval to facilitate easy absorption of TZ solution was done. Viability (%) was recorded for every 1 hour upto 6 hrs and finally after 24 hours. The seeds were washed 2-3 times with distilled water and 3 slides were prepared for every sample as 3 replications. 5 microscopic fields per slide were considered for counting viable seeds, but not less than 200 seeds in total. After collection, some capsules/seeds were dried in ambient condition wrapping with butter paper for one month. Pre-storage seed viability testing was done following standardized method. The capsules were cut open and seeds from 2-3 capsules were mixed properly and stored in already oven dried test tubes. A little amount of CaCl, (1:50:: desiccant: seeds) was put at the bottom of test tube and a small amount of cotton was placed over it. Then the seed samples were wrapped with butter paper and were placed over cotton, test tubes were plugged with cotton and closed with parafilm. The test tubes were stored under ambient condition (room temperature), in culture room (25±20C) and in fridge at 4°C. Stored seeds were evaluated at 90, 180 and 270 days of storage (DOS).

RESULTS AND DISCUSSION

In the study of Tetrazolium test for concentration and duration in three tropical orchid species namely, *Phaius tankervillae, Rhyncostylis retusa* and *Aerides odoratum,* different species showed different response in concentration as well as duration of treatment (Table 1).

Considering more or less stable observation and time and chemical economy, 24 hrs treatment of Tz solution was suitable for *P. tankervillae*, whereas for *R. retusa* and *A. odoratum*; it was suitable for 3 hrs and 2 hrs treatment respectively at 0.5% Tz solution.

The response of the three orchid species varied to storage conditions (Table 2). Fridge storage (4°C) was better for *P. tankervillae,* and *R. retusa.*. However, the loss of viability in all the storage conditions was very fast in case of *R. retusa*. Since seeds of *P.*

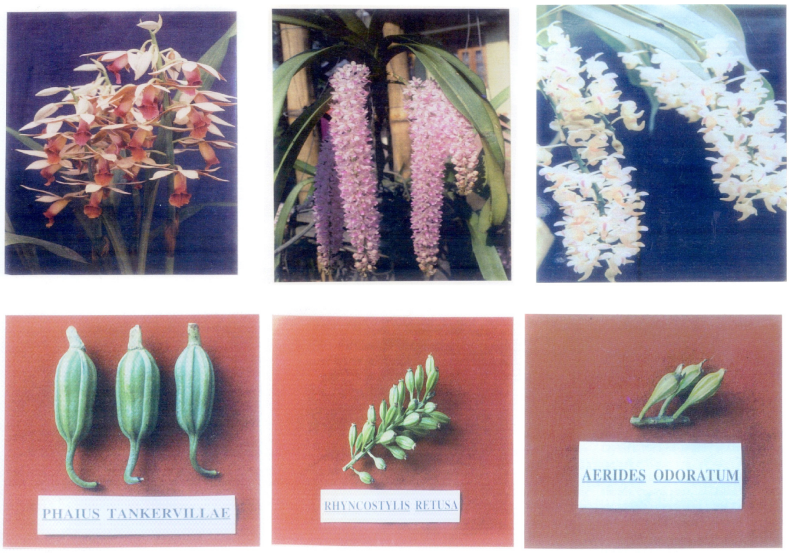
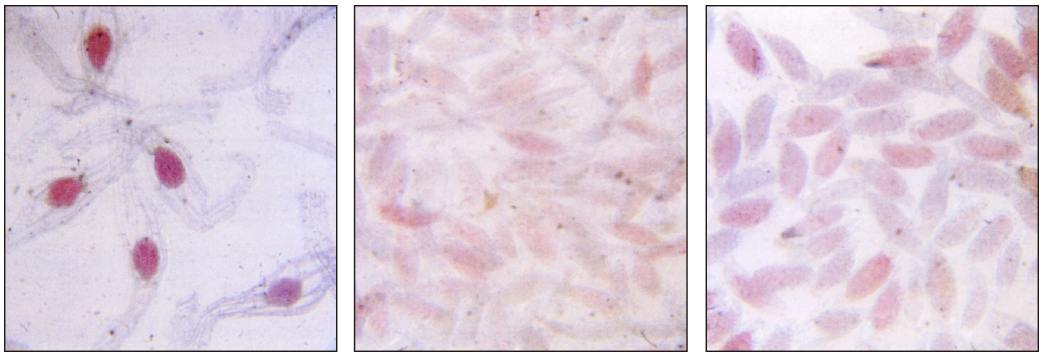


Table1. Standardization of Tz- concentration and duration of treatment for viability testing of three tropical orchids

Species	Concentration (%)	Viability Percentage						
		1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	24 hrs
Phaius tankervilliae	0.1	-	-	-		9.50	28.36	69.14
	0.5	-	-	-	Just start	61.10	82.50	88.49
	1.0	-	-	-	Just start	71.46	83.50	89.68
Rhyncostylis retusa	0.1	8.50	36.30	75.00	96.70	97.00	97.00	96.90
	0.5	27.20	82.50	95.50	96.33	95.25	95.00	95.34
	1.0	39.41	91.70	98.32	96.25	97.20	100	100.00
Aerides odoratum	0.1	29.12	80.30	90.70	90.00	92.35	89.95	92.70
	0.5	76.43	92.21	98.00	97.52	97.20	98.58	99.20
	1.0	80.31	97.00	100.00	99.15	98.30	99.00	100.00



(a) Phaius tankervilliae *(b) Rhyncostylis retusa* *(c) Aerides odoratum*

Tetrazolium Seed Viability testing observation under microscope

tankervilliae could be stored as well as compared to other two species, it may be due to its genetic potentiality in exerting more mechanical restriction against loss of seed viability and/or due to high suberin deposition causing less permeability of the seed coat. These findings were in conformity with the observations of [5 & 6], where varied response of orchid seeds during storage under different condition was recorded.

The response of orchid species *Aerides odoratum* could not be ascertained due to low seed viability at the time setting seed under storage after similar one month exposure in ambient room temperature. This may be due to lesser storability property of the species.

Varying seed coat impermeability due to differences in suberin deposition was specific to genetic constitution of individual species. High suberinization on the seed coat led to less permeable nature of water [7]. It is also noted the differences in permeability of the seed coat amongst Western European and tropical orchids.

Such specificity could be revealed by erratic trend in manifesting seed viability over different Tz concentration and duration of treatment.

It is evident from the study that viability testing through Tz solution is suitable for orchid sps. under study, duration is specific to individual sps viz. *Phaius tankervilliae, Rhyncostylis retusa and Aerides odoratum* - 24 hrs treatment of Tz solution was suitable for *P. tankervilliae*, whereas for *R. retusa* and *A. odoratum*; it was suitable for 3 hrs and 2 hrs treatment respectively at 0.5% Tz solution.

Out of three storage conditions, fridge storage (4°C) was better for *P. tankervilliae,* may be due to its genetic potentiality in exerting more mechanical

Table 2. Change in seed viability during storage

Species	Conditions	At the time of storage	90 DOS (%)	180 DOS (%)	270 DOS (%)
Phaius tankervilliae	RT	84.35	50.28	11.33	0.03
	CR		53.03	15.00	0.07
	FR		61.78	23.17	12.87
Rhyncostylis retusa	RT	80.49	30.32	0.00	0.00
	CR		32.80	0.06	0.00
	FR		40.64	0.20	0.00
Aerides odoratum	RT	35.00	0.00	0.00	0.00
	CR		0.00	0.00	0.00
	FR		0.00	0.00	0.00

Note: RT= Room temperature; CR= Culture room (25±2°C); FR= Fridge (4°C); DOS= Days of storage

restriction against loss of seed viability and/or due to high suberin deposition causing less permeability of the seed coat.

In vitro propagation of these orchids through seeds will help to retain most of the variable genotypes, which otherwise lost since only a small fraction of seeds (0.2-0.3%) germinate in nature.

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