

Effect of Different pH on *In vitro* Seed Germination of *Vanda tessellata* (Roxb.) Hook. Ex. G an Endangered Medicinal Orchid .

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Abstract

The present investigation deals with a study of *In-vitro* seed germination of an endangered medicinal orchid *Vanda tessellata* at different pH (3.5, 4.5, 5.5, 6.5). Immature seeds obtained from green pods were successfully germinated on basal media MS without various combinations of growth hormones. Highest seed germination (95±0.17%) were observed at 5.5 pH, whereas the medium which is adjusted at 3.5 and 6.5 pH showed poor seed germination.

Keywords:- *Vanda tessellata*, seed germination

1. Introduction

Vanda tessellata (Roxb.) Hook. Ex. G. is an endangered medicinal plant belong to the family Orchidaceae. Family orchidaceae is well known as ornamental plant which have attractive flower and high purchase prices. The Orchidaceae were reported that has belonging a lot of species grew in the world, consisting of 35,000 species and 800 genera **Singh et.al., (2007)**. The orchids were observed to produce abundant seeds in their fruit capsules. In previous studies it was reported that orchid produced 1,300 to 4 million seeds per capsules (**Pierik, 1987**). As the seeds do not have sufficient reserve food material (lacks endosperm) to take care of the growth of embryo during germination they have to depend on some external source for nutrients so as to make their undifferentiated embryo to develop into a protocorm. *Vanda tessellata* (Roxb.) Hook. Ex. G. is a species of orchid occurring from the Indian subcontinent to Indochina. It is an epiphytic orchid, 30-60 cm high, with leafy stem. Leaves are thickly coriaceous, recurved, plicate, obtuse keeled. Flowers are greenish yellow, mottled with brown on the mid lobe of lip with purple caruncles (**N.S. Chauhan, 1999**). Petals yellow with brown lines and white margins, shorter than the sepals, Lip 16 mm long, bluish, dotted with purple. Capsules 7.5-9 cm long, narrowly clavate-oblong with acute ribs. Paste of its leaves is used as application in fevers. It is ingredient of *Rasna Panchaka Quatha*, Ayurvedic formulation used in the treatment of arthritis and rheumatism. Expressed juice of the leaves is used in the treatment of otitis media. The root is used as antidote against scorpion sting and remedy for bronchitis (**N.S. Chauhan, 1999**). In the present study, an attempt was made to have a mass clonal propagation of *Vanda tessellata* (Roxb.) Hook. Ex. G. the rare species of the genus in Madhya Pradesh within a short span of time, the aim was to study the effect of pH on seed germination in MS media.

2. Material and method

The immature pods of *Vanda tessellata* were collected from the forest around Bhopal. First the dry petals attached to the green pods were removed, then the pods were washed thoroughly by using running tap water for (30min), and were further treated with an antifungal agent (**Bivastin**) for 1 hour and with detergent for 10 min. The pods were surface sterilized by immersion in 0.1% mercuric chloride (HgCl₂) solution for 25-30 min in laminar air flow followed by throw wash in distilled water. Then the pods were dipped quickly in 70% alcohol and flamed over a spirit lamp. Each pods were then transferred to a sterile petri plate. Then the pods were cut longitudinally into two halves using sterile scalpel, and the seeds together with cottony fibers in between were scooped out. After careful separation of the seeds from the fibers, the seeds were transferred onto MS (**Murashige and Skoog, 1962**). One set of each seed culture was maintained at pH (3.5, 4.5, 5.5, 6.5). The Basal medium was amended with 3 % (w/v) sucrose. The pH was adjusted from 3.5 to 6.5, prior to adding 0.8% agar. The media was autoclaved at a temperature of 125°C at pressure of 15 psi for 15-20min in 100ml conical flasks.

All such operation was done within a Laminar Air Flow Cabinet. The culture bottles were incubated in culture room at 25 ± 20°C under 16 hrs. photoperiod of approximately 2,500 flux light intensity from cool fluorescent tubes.

3. Result and discussion

The response of seed germination was noted by observing the colour change and shape of the seed. During germination, embryos were seen to emerge from the seed coat as yellow to creamy structure. The culture attained sperule shape (Fig.a) and soon after start germinating within 60 to 120 day (Fig. b &c). The onset of seed germination by immature seeds at different pH (3.5, 4.5, 5.5, 6.5,) were recorded periodically after the day of initial inoculation (table 1 & Fig.1).

Highest seed germination ($95 \pm 0.17\%$) were achieved in MS medium adjusted at 5.5 by immature seeds (Fig.c). In our present study MS media adjusted at pH 5.5 is found suitable over 3.5, 4.5, 5.5, 6.5 for seed germination. The culture on MS media adjusted at pH 5.5 germinated and survived beyond three month of culture period.

Similar results were observed when the seeds of *D.chrysanthum* and *S.pallidus* were inoculated in media adjusted at 5.0 pH. In the earlier studies, *D.nobile* germinated better within a pH range 4.0-5.0 (Quednow, 1930; Ito, 1995). The present investigation suggests the specificities of pH requirements during seed germination of orchids.

4. Conclusion

From the above findings, it may be concluded that MS medium adjusted at pH 5.5 is best for seed germination of *Vanda tessellata* orchid in comparison to the pH adjusted at 3.5, 4.5, 5.5, 6.5. All this data there by suggests that the pH plays a very important role in *In-vitro* seed germination. Hence pH plays a very important role for shortening the growth period and rapid propagation of *Vanda tessellata* a medicinally important endangered orchid.

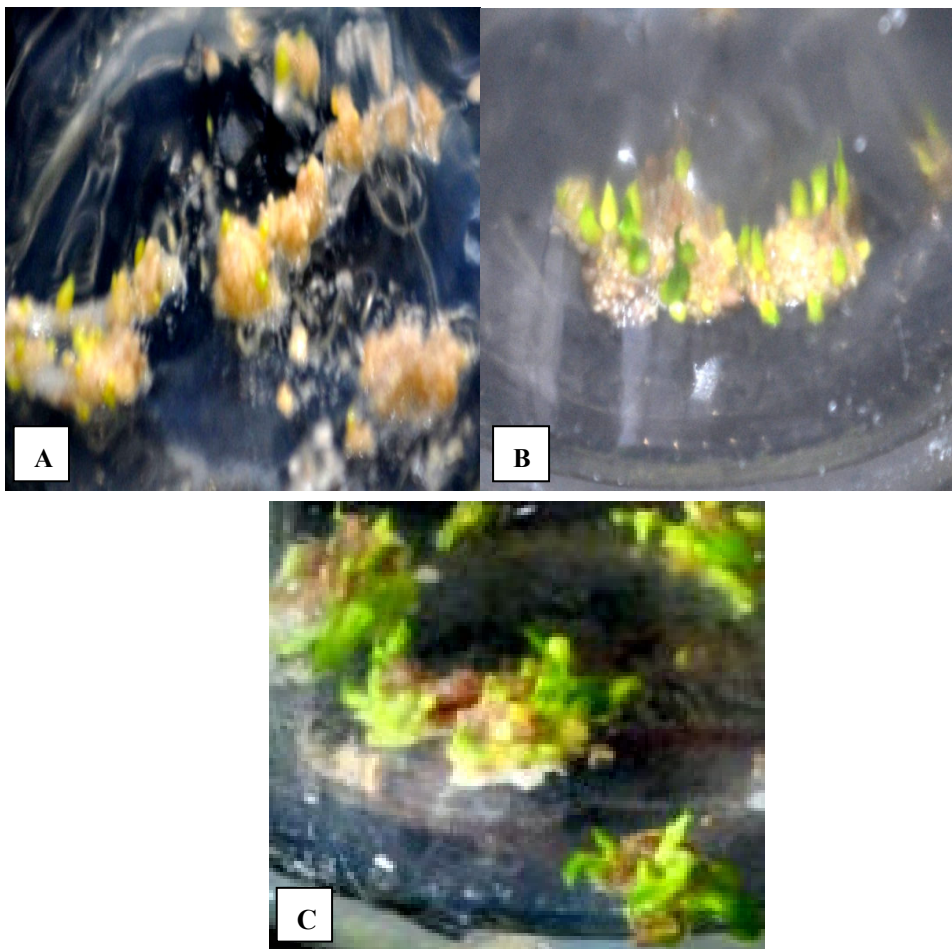
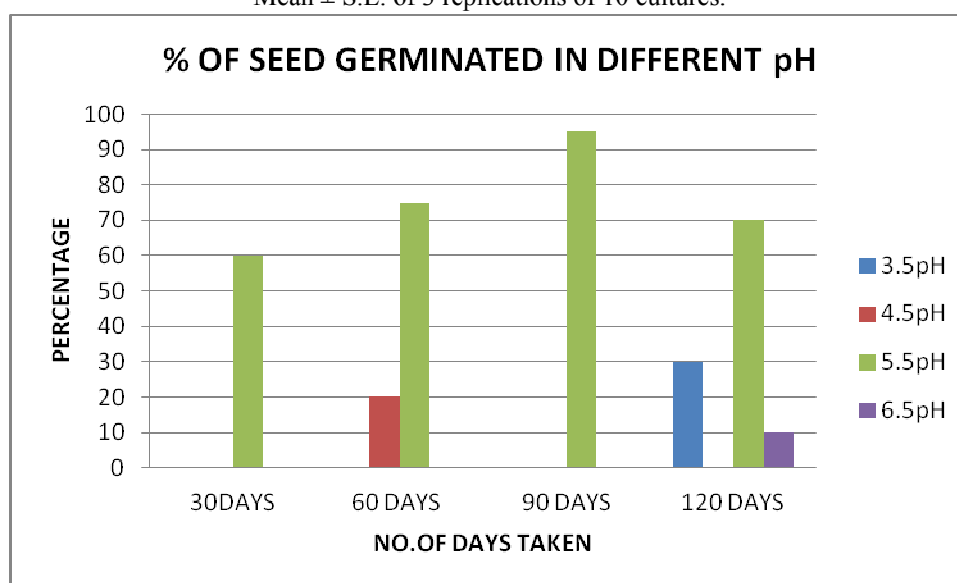


Fig1:- Seed germinated in MS media adjusted at pH 4.5 in 60 days, B. seed germinated in 30 days at pH 5.5, c. Seed germinated in MS media adjusted at pH 5.5 in 90 days.

Table.1. Seed germination potential of *Vanda tessellata* (Roxb.) Hook. Ex. G at different pH.

% seed germination in mean \pm SE				
MS media				
Different pH	30 days	60 days	90 days	120 days
3.5	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	30 \pm 0.12
4.5	0.0 \pm 0.0	20 \pm 0.18	0.0 \pm 0.0	0.0 \pm 0.0
5.5	60 \pm 0.27	75 \pm 0.20	95\pm0.17	70 \pm 0.10
6.5	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	10 \pm 0.19

Mean \pm S.E. of 3 replications of 10 cultures.

**Fig 1. Seed germination potential of *Vanda tessellata* (Roxb.) Hook. Ex. G at different pH (3.5,4.5,5.5,6.5)**

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