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Impact of temperature and pH Variation on in-vitro Protocorm formation of *Vanda tessellata* (Roxb.) Hook. ex G.Don an endangered medicinal Orchid

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Abstract

The present research work is carried out to study the In-vitro protocorm formation of an endangered medicinal orchid Vanda tessellata at different temperature(15°C, 20°C, 25°C, 30°C) and pH (4.5, 5.5, 6.5,7.5) . Immature seeds obtained from green pods successfully shown protocorm formation on basal media MS without various combinations of growth hormones. Highest protocorm formation (94±0.17%) were observed at temperature 20°C and pH 5.5 , whereas the medium which is adjusted at temperature 15°C, 25°C, 30°C and pH 4.5, 6.5, 7.5 showed poor protocorm formation.

Keywords: *Vanda tessellata*, seed germination, protocorm, In-vitro.

Introduction

ORCHIDS, the most beautiful flowers in God's creation, comprise a unique group of plants. Orchidaceae were reported that has belonging a lot of species grew in the world, consisting of 35,000 species and 800 genera[1]. Orchids exhibit an incredible range of diversity in size, shape and colour of their flowers. Among the various orchids, *Vanda tessellata* (Roxb.) Hook. ex G.Don is one of the most ethno-botanically important taxa. It is an endangered medicinal plant belongs to the family orchidaceae. It is an epiphytic orchid, 30-60 cm high, with leafy stem. Capsules 7.5-9 cm long, narrowly clavate-oblong with acute ribs. Paste of its leaves is used as application in fevers. It is used in the treatment of arthritis and rheumatism. The root is used as antidote against scorpion sting and remedy for bronchitis[2]. 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. However *Vanda tessellata* in natural habitats are quickly diminishing due to heavy deforestation, urbanization, utilization of land for agriculture and over-exploitation of agro-resources for commercial purposes. To save this diverse orchid species from extinction, in vitro culture techniques have been utilized to propagate them[3]. In the present study, an attempt is made to have a mass clonal propagation of *Vanda tessellata* an endangered species within a short span of time; the aim is to study the effect of temperature and pH on protocorm formation in MS media.

Material and Methods

The immature pods of *Vanda tessellata* where collected from the forest around Madhya Pradesh. Firstly the dry petals attached to the green pods were removed, then the pods were washed properly by using running tap water for (30min), and was further treated with an antifungal agent (Bivastin) for 1 hour and with detergent for 10 min. The pods were surface sterilized by soaking in 0.1% mercuric chloride ($HgCl_2$) solution for 25-30 min in laminar air flow thereafter by throw washing in distilled water. Then the pods was 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 equal 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[4]. One set of each seed culture was maintained at temperature (15°C, 20°C, 25°C, 30°C) and pH (4.5, 5.5, 6.5,7.5). The Basal medium was amended with 3 % (w/v) sucrose. The temperature was adjusted from 15°C to 30°C and pH was adjusted from 4.5 to 7.5, 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[5]. 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.

Results and Discussion

The response of protocorm formation 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 (Figure-2(a) and soon after it starts protocorm formation within 60 to 120 day (Figure-2b). The onset of protocorm development by immature seeds at different temperature and pH where recorded periodically after the day of initial inoculation (table-1).

Highest protocorm formation (94±0.17%) was achieved in MS medium adjusted at temperature 20°C and pH 5.5. In our present study MS media adjusted at temperature 20°C and pH

5.5 is found suitable over the temperature 15°C, 25°C, 30°C and pH 4.5, 6.5, 7.5 for protocorm formation (figure-1).

Similar results were observed when the seeds of orchid *Cattleya mossiae* and *Lealia purpurea* germinated well when adjusted at 20°C[6]. In the earlier studies, *D.nobile* germinated better within a pH range 4.0-5.0[7]. The present investigation suggest the specificities of temperature and pH requirements during In-vitro protocorm formation of orchid.

Conclusion

From the above findings, it may be concluded that MS medium adjusted at temperature 20°C and pH 5.5 is best for protocorm formation of *Vanda tessellata* orchid in comparison to the temperature adjusted at, 15°C, 25°C, 30°C and pH adjusted at 4.5,6.5,7.5. Hence all this data there by suggests that the temperature and pH plays a very important role in In-vitro protocorm formation of *Vanda tessellata* a medicinally important endangered orchid.

Table-1
Protocorm formation of *Vanda tessellate* (Roxb.) Hook.ex G. Don at different temperature and pH

%Protocorm formation in mean ± SE					
MS media					
Different Temp.	**Different pH**	**30Days**	**60Days**	**90Days**	**120Days**
15°C	4.5	0.0±0.0	0.0±0.0	0.0±0.0	30±0.12
20°C	5.5	0.0 ± 0.27	75±0.20	**94±0.17**	80.0 ±0.10
25°C	6.5	0.0±0.0	20±0.18	0.0±0.0	0.0±0.0
30°C	7.5	0.0±0.0	0.0±0.0	0.0±0.0	1.0±0.19

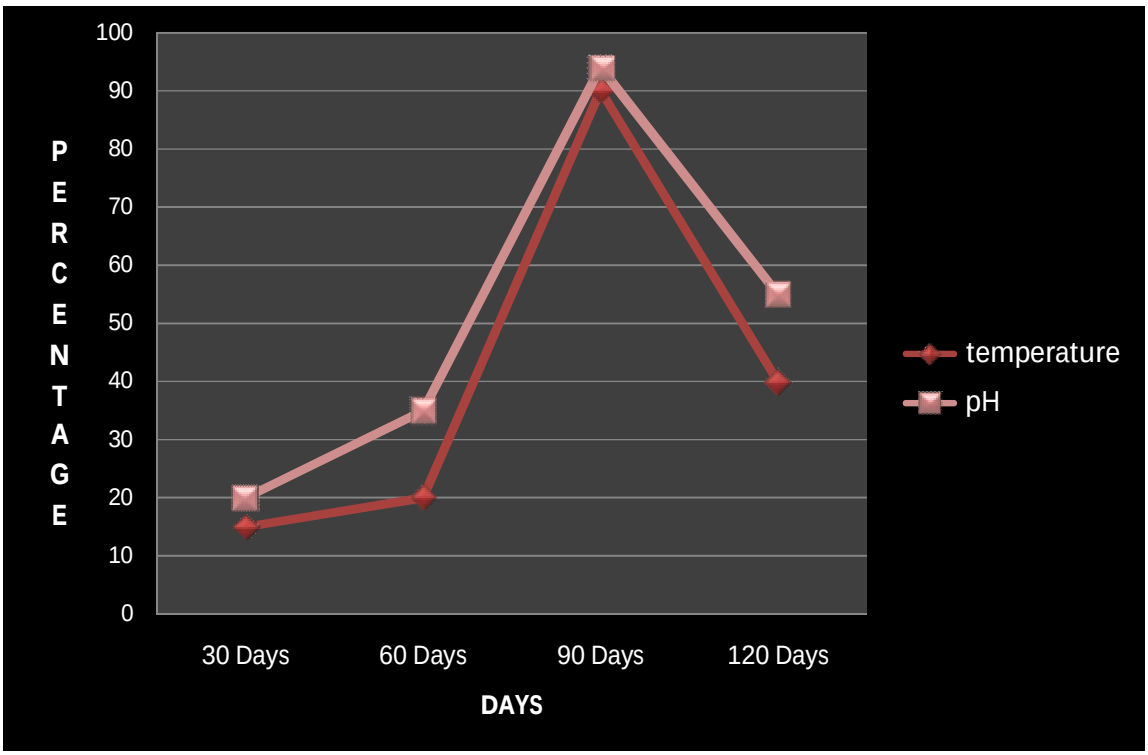

Figure-1
Protocorm formed at different temperature and pH.

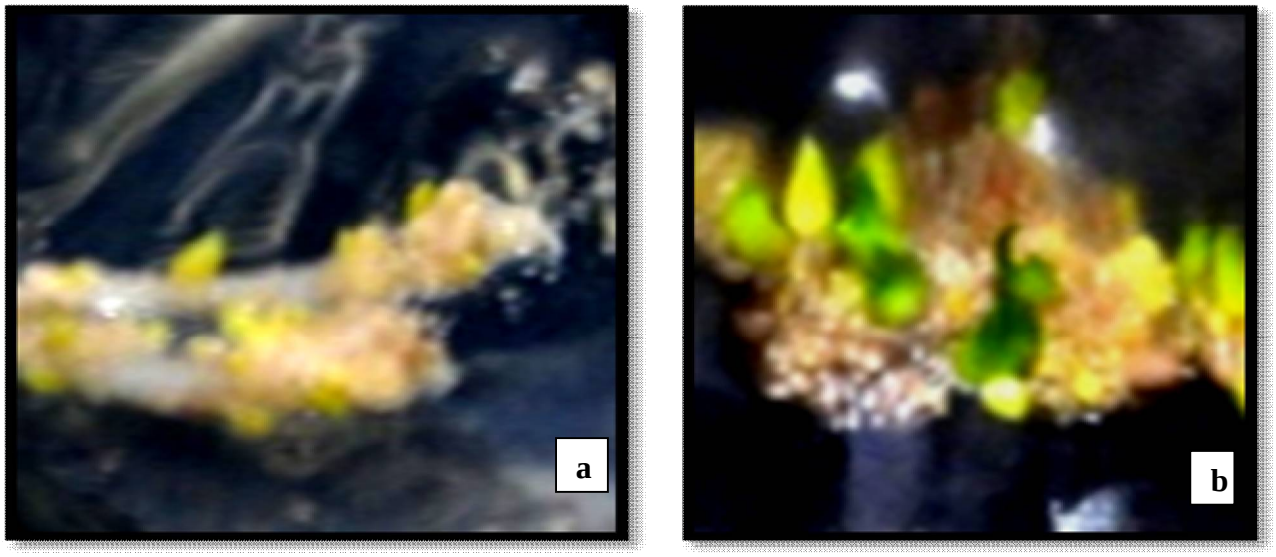

Figure-2
(a)PLB'S formed in MS media adjusted at temperature 20°C and pH 5.5 in 90 days,
(b) PLB'S formed after 120 days in MS media adjusted at temperature20°C and pH 5.5

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