# Effect of different media on *In-vitro* seed germination and protocorm formation of *vanda tessellata* (Roxb.) Hook. Ex. G. Don an endangered medicinal orchid

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**Abstract:** The present investigation deals with a study of *In-vitro* seed germination and protocorm formation of an endangered medicinal orchid *Vanda tessellata* by using four different media. Immature seeds obtained from green pods were successfully germinated on four basal media (BM), Kn C, VW, MS and RT without various combinations of growth hormones. The protocorm development was hastened up with the minimum period required for the onset of germination on defined medium. The seed germination was assessed at up to 65% on KnC, 40% on VW, 95% on MS and 30% on RT by whereas protocorm formation was assessed at up to 50% on KnC, 37% on VW, 90%on MS and 20% on RT. Highest seed germination (95%) and protocorm formation (90%) were achieved in MS medium.

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**Keywords:** *Vanda tessellata,* protocorm ,seed germination, micronutrients.

**Abbrevations:** BM - (Basal media), KnC - (Knudson, C)**,** MS **-** (Murashige and Skoog)**,** VW - (Vacin and Went )**,** and RT (Raghavan and Torrey)**.**

## 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. Large quantity of Orchidaceae seeds will germinate *in-vitro* condition if we cultured onto appropriate germination medium. *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 different culture media on its seed germination and protocorm formation.

## 2. Material and method

For asymbiotic seed germination, different basal media, *viz.,* KnC ( **Knudson, 1946),** MS **(Murashige and Skoog, 1962)** ,VW **(Vacin and Went, 1949)** and RT **(Raghavan and Torrey, 1964)** without any growth hormones were used for the present investigation. The immature pods of *Vanda tessellata* where 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 ($Hgcl_2$) solution for 25-30 min in laminar air flow followed by thorough 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 different media. One set of each seed culture was maintained on Kn C, VW, MS and RT basal media . The Basal medium was amended with 3 % (w/v) sucrose. The pH was adjusted between 5.5-5.9, the media were adjusted 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. Most of the seeds were embroynate and were found swollen within three or four week. During germination, embryos were seen to emerge from the seed coat as yellow to creamy structure (Fig.A). The culture attained sperule shape and soon after developed as protocorm within 60 to 120 day(Fig.B & C). The onset of seed germination and the formation of protocorm by immature seeds on four basal media KnC, VW, MS and RT media were recorded periodically after the day of initial inoculation (table1&2).

Highest seed germination (**95±0.13%**) and protocorm formation (**90±0.17%**) were achived in MS medium by immature seeds . In our present study MS media is found suitable over KnC, VW and RT for seed germination and protocorm formation. The culture on MS media were healthy protocorm and survived beyond three month of culture period.

Similar results were observed in the seeds of ***Cymbidium mastersii*** and ***C. ebernium*** when germinated on KnC basal medium, **Prasad and Mitra ( 1975). Mitra (1971**) reported that the seeds of ***Arundina*** however did not germinate on KnC basal media but they responded well on VW and RT basal media.Whereas **Sinha S K et al.,(1998)** reported that KnC, VW and MS is suitable for ***Aerides rosea.*** These previous studies reveals that no single nutrient medium is universally suitable for seed germination of all or most of the orchid taxa.

## 4. Conclusion

From the above findings, it may be concluded that MS medium is best for seed germination and protocorm formation of *Vanda tessellata* orchid in comparison to that of other media by immature seeds. All this data there by suggests that the nutritional requirement in In-*vitro* is species –and/ or genera-specific. Hence media plays a very important role for shortening the growth period and rapid propagation of *Vanda tessellata* a medicinally important endangered orchid.

**Table 1. Seed germination potential of *Vanda tessellata* (Roxb.) Hook. Ex. G.Don in different medium (Kn C, VW, MS, RT).**

| % seed germination in mean ± SE | | | | |
|---|---|---|---|---|
| Media | 30 days | 60 days | 90 days | 120 days |
| Kn C | 0.0±0.0 | 54±0.21 | 65±0.14 | 73±0.12 |
| VW | 0.0±0.0 | 0.0±0.0 | 35±0.19 | 40±0.15 |
| MS | 60 ± 0.29 | 75±0.19 | 95±0.13 | 70 ±0.11 |
| RT | 0.0±0.0 | 0.0±0.0 | 20±0.30 | 30±0.19 |

Mean ± S.E. of 3 replications of 10 cultures.

**Table 2. Protocorm formation of *Vanda tessellata* (Roxb.) Hook. Ex. G.Don in different medium (Kn C,VW, MS, RT).**

| % protocorm formation in mean ± SE | | | | |
|---|---|---|---|---|
| Media | 30 days | 60 days | 90 days | 120 days |
| Kn C | 0.0±0.0 | 0.0±0.0 | 50±0.19 | 65±0.12 |
| VW | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 37±0.26 |
| MS | 0.0±0.0 | 70±0.18 | 90±0.17 | 80 ±0.20 |
| RT | 0.0±0.0 | 0.0±0.0 | 00±0.0 | 20±0.30 |

Mean ± S.E. of 3 replications of 10 cultures

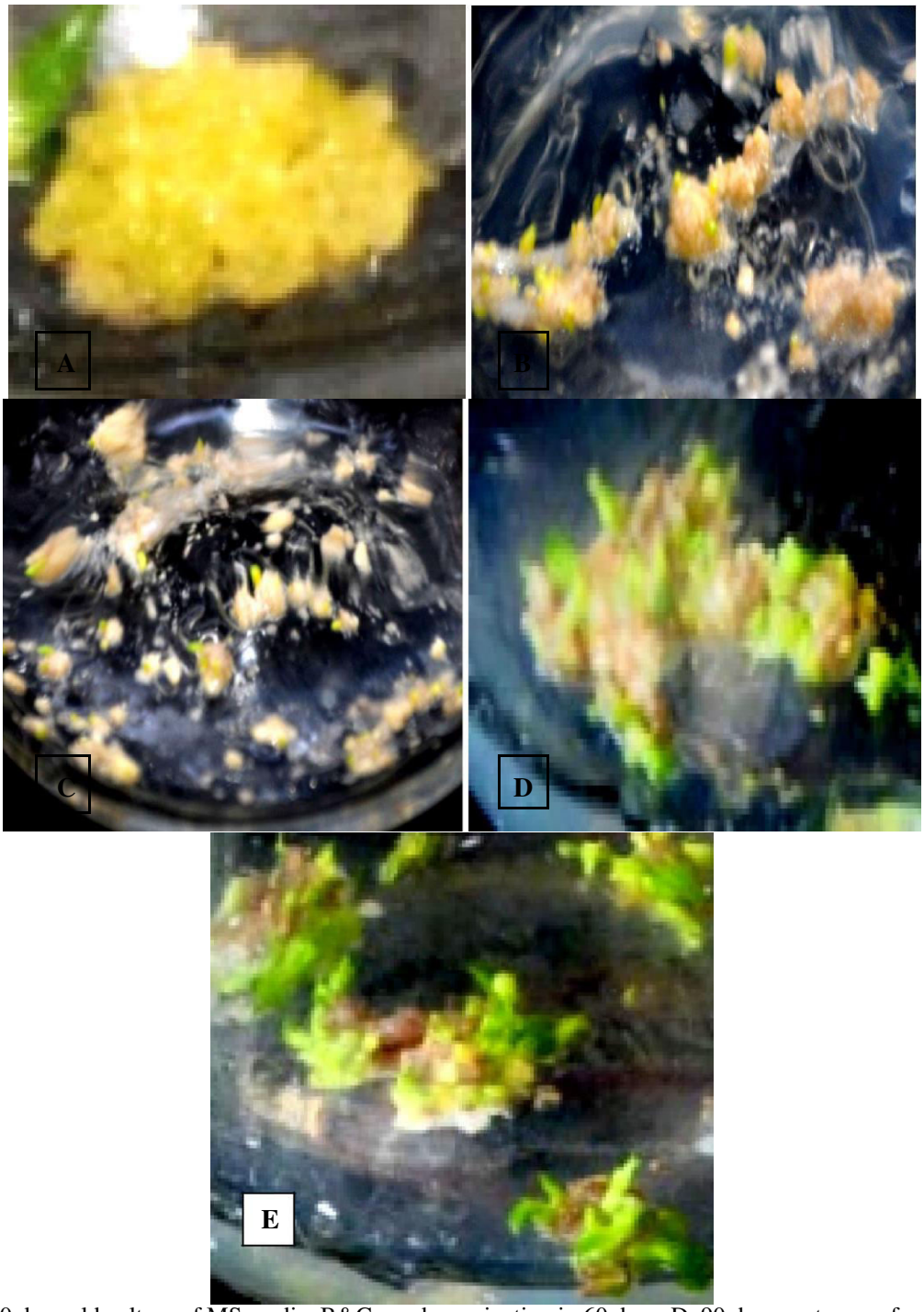


**Fig1:-** A. 30 days old culture of MS media, B&C. seed germination in 60 days, D. 90 days protocorm formation in 90 days, E. 120 days maximum protocorm formed in MS media.

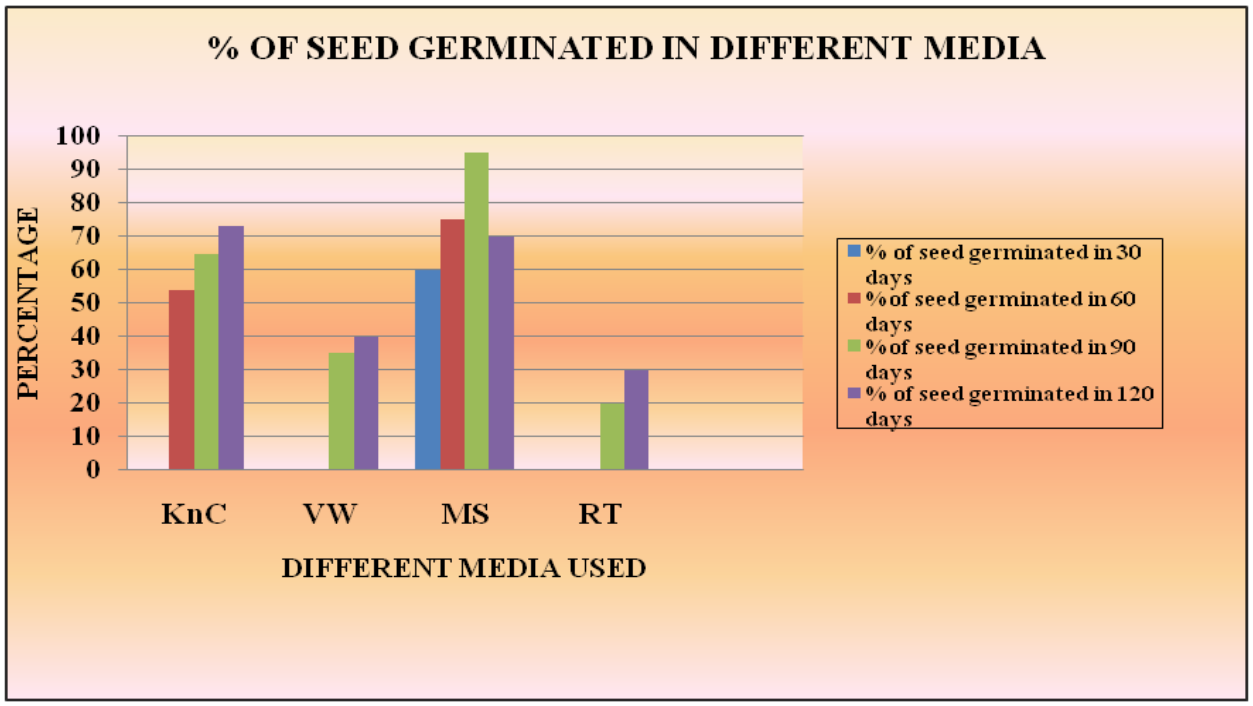


**Fig .2. Seed germination potential of *Vanda tessellata* (Roxb.) Hook. Ex. G.Don in different medium (Kn C, VW, MS, RT).**

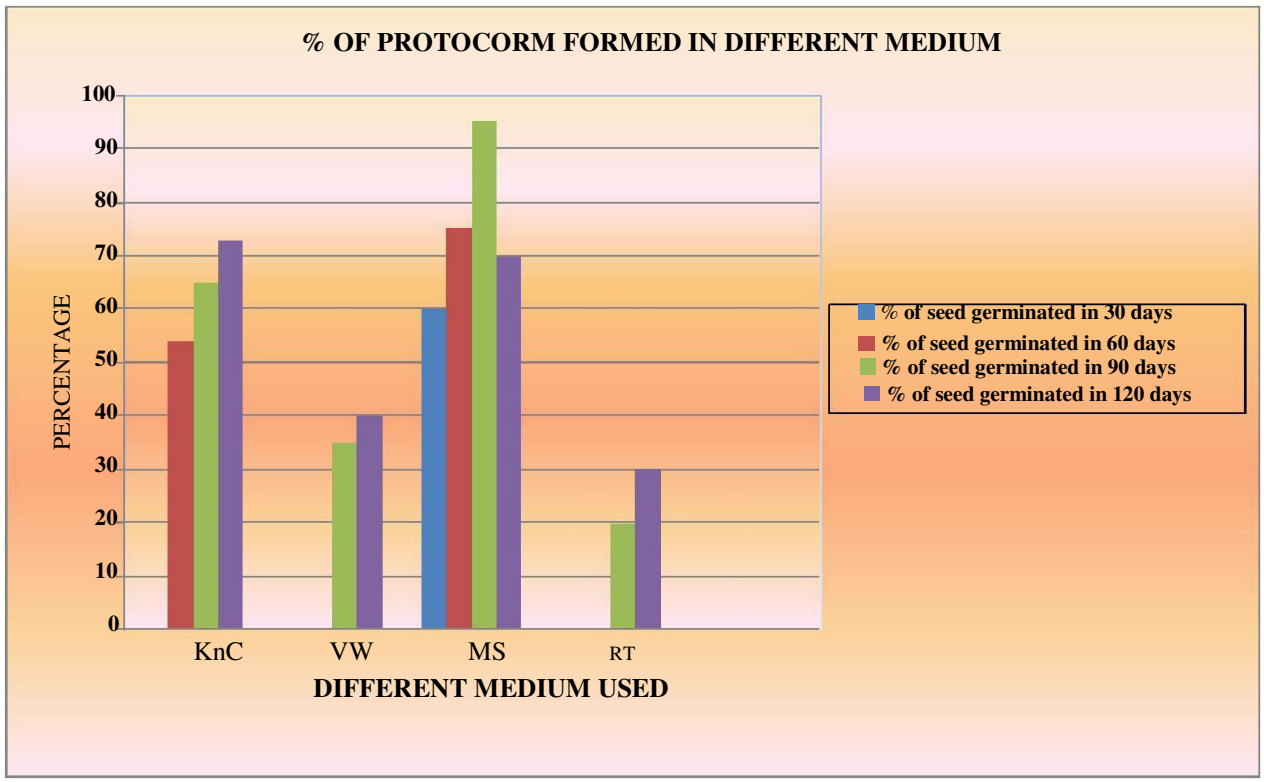


**Fig .3. Protocorm formation of *Vanda tessellata* (Roxb.)Hook.Ex.G.Don in different medium (Kn C,VW,MS,RT).**

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