SUMMARY

*Vanda coerulea* (family - Orchidaceae) popularly known as 'Blue vanda' is an endangered orchid species found wild in the states of Meghalaya an Arunachal Pradesh of India. The Blue Vanda is one of the most highly priced orchid species in the International market. The species has already been brought under Wild Life Protection Act of India banning its collection and trade.

Natural propagation of blue Vanda is a slow process. So an attempt has been made to standardize a tissue culture protocol for mass propagation of this important orchid species. Along with mass propagation, effect of gamma irradiation and chemical mutagen EMS treatment were studied on the in vitro germination and growth of *Vanda coerulea*. The detailed of the work done and results obtained are summerised below

- 280 -310-days old capsule of *Vanda coerulea* was used for the mass propagation experiment.
- A comparative study among different culture media viz. Knudson, Gamborg's, Lindermann, Vacin & Went for standardization of the basal medium was conducted to test their performance in percentage of seed germinations and days to germination. Knudson media proved to be best requiring minimum time(24 days) for seed germination and highest percentage of germination(90%), followed by a comparable performance by Gamborg's medium (40 days and 55%), Lindermann (45 days and 40%) and Vacin & Went (60 days and 25%).
- Further the basal Knudson medium was modified into 4 different modified media viz K₁, K₂, K₃, K₄ ( table no. 4 ) which were also tested for days to germination and percentage of germination. Out of these 4 modified media, K₂ performed the best taking minimum time for germination (18 days) and germination percentage (95%), followed by K₁, K₃, K₄.
- Different growth regulator treatments viz. NAA (0.5 – 3.0 mg/l) + BAP (0.1 – 0.3 mg/l ) (Table 5) combinations, IAA (0.5 – 3.0 mg/l) + Kinetin (0.1- 0.3 mg/l) (Table 7) combinations and IBA (0.5 – 3.0 mg/l) + 2 iP (0.1- 0.3 mg/l)(Table 9) combinations were tried for response of protocorm & seedling development.
Among the twelve different combinations of NAA and BAP, highest response of protocorm (88%) was recorded in NAA (2.0 mg/l) and BAP (0.2 mg/l) (Table 5). The same combination took minimum time (2.75 weeks) for first leaf emergence, with highest shoot length (3.57 cm) and leaf number (7.00 nos.) (Table 7) in 20 weeks old culture. Early root emergence (4.70 weeks) with healthy long roots (5.43 cm) and (5.75 nos.) (Table 6) were recorded in the same NAA (2.0 mg/l) + BAP (0.2 mg/l) combination.

Effects of IAA (0.5-3.0 mg/l) and Kinetin (0.1-0.3 mg/l) (Table 7) in twelve different combinations was studied. The response of protocorm was comparatively less than NAA + BAP combinations. Among these IAA + Kinetin combinations, IAA (1.0 mg/l) + Kinetin (0.1 mg/l) (Table 8) showed highest response (65%), which also showed early leaf initiation (7 weeks), longest shoot (1.53 cm) and leaf number. However, better root characters were obtained in IAA (1.0 mg/l) + Kinetin (0.3 mg/l).

Response of protocorm in IBA (0.5-3.0 mg/l) + 2iP (0.1-0.3 mg/l) (Table 9) combinations were comparatively much lower than NAA + BAP or IAA + Kinetin. Here highest response (54.25%) was recorded in IBA (2.0 mg/l) + 2iP (0.3 mg/l). The same combination showed better root characters, length (2.18 cm) and number (2.23). However shoot characters were best in IBA (0.5 mg/l) + 2iP (0.3 mg/l).

Among the four different potting materials tried for hardening of in vitro raised Vanda seedlings, moss had higher survival rate (90%) (Table 11) than the other material of coconut husk, brick and charcoal or sand and vermiculite mixture. Again community plantation of 5-6 seedlings in clumps had higher survivility than single plantation in hardening. After one month of successful hardening period, the seedlings were transferred to orchidarium (agroshed).

Among twenty different doses of gamma irradiations (0, 10, 20, 30, ..., 200 Krad) given on twenty days old protocorms of Vanda coerulea. Survivility of protocorms were found upto 5 days in 80 Krad, 10 days in 60 Krad (Table 13), 15 days in 50 Krad. The treated protocorms in 90 Krad and above were turned brown within five days. Protocorms treated in 10 to 40 Krad survived upto 4 months with different survivility rate.

The survived protocorm after 4 months were transferred to K2 modified media and the growth parameters were found to be decreasing with
increasing doses of gamma irradiated protocorms. Highest shoot length, numbers of leaf, root numbers and length were observed in control and the irradiated protocorms developed small shoots and roots.

- Among the various doses of EMS (0.1, 0.2, 0.3...,1.0%) (Table 15) on protocorms of *Vanda coerulea* for 30 minutes the germination percentage of the response of protocorm decreased with increase in concentration of EMS. While in control it was 100% germination. At 0.4% of EMS. There was 50% germination and beyond 0.8% there was no germination at all.

- Irradiated plantlets were hardened for a month and transferred to orchid house. 3 months after hardening, the irradiated plantlets were observed to be shorter with small leaves as compared to normal unirradiated plantlets.

- The survived protocorms from 0.1 to 0.7 % EMS concentration were transferred to fresh K$_2$ + NAA (2.0 mg/l) + BAP (0.2 mg/l). Amongst the different dose, protocorms of 0.1% concentration had more number of leaves as compared to control and other treatments (Table no.15). Leaf number, leaf length, root number and root length was decreasing with increasing concentration of EMS.

- The EMS treated *Vanda* plantlets were hardened for a month. There was no difference in growth parameters with the untreated plantlets.