OBSERVATIONS
For successful micropropagation, it is well known that the orchids require at least three different stages of explant / callus / PLB / plantlet transfers to different media. Each medium with its own distinctive composition of nutrients, growth regulators etc., has characteristic influence on morphogenetic process, thus reducing the time to yield the required, transplantable plantlets. In this study, the generally employed three step process is extended to a five step process i.e., from the explant stage to the regenerated transplantable plantlet stage, five different media combinations were used. Further, to evaluate the relative merits of commonly used media in orchid tissue culture, these five different stages were repeated with VW, MS and KC media. These three media were selected for the study after screening the literature on in vitro study of about 260 orchids, which has shown that of the thirty four media compositions used, more than 65% of the orchids have been studied using these three media (see Table 1).

In vitro morphogenesis was monitored at regular intervals (twice a week) and recorded. In vitro behaviour of different explants used in the study are recorded separately for each of the orchid. In all, six explant sources have been used in this study viz., shoot apex, inflorescence, leaf, root, node and internode. In Phalaenopsis however, shoot apex was not used in view of the monopodial nature of the orchid and excision of a shoot tip means death of the plant itself.
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Before initiating the *in vitro* studies a careful explant screening and selection method was followed to ensure a higher rate of regeneration. Plants from both commercial nurseries as well as regenerated source were used to obtain the required explants. It was found that the plants from the nurseries needed an in-house maintenance for at least fifteen days for yielding good quality explants.

The following are the five stages under which morphogenesis of each explant under appropriate media conditions was studied:

1. Induction of callus or PLBs
2. Multiplication of callus or PLBs
3. Regeneration of plantlets
4. Multiplication of plantlets and
5. Growth and rooting of plantlets

**INDUCTION OF CALLUS:** *Time taken for visual appearance of callus*

Callus was induced from explants in all the three media mentioned earlier with varied concentration of growth regulators. The data on the callus generated was studied with regard to requirement of time for callus initiation, percentage of callusing, degree of callusing and degree of multiplication of callus.
For purposes of initiation of callus the duration required for the visual identification of the whole or part of the explant used for dedifferentiation was taken into account. Thus, the number of cells dedifferentiated could vary at this initial stage. In general, for all the four orchids, the initiation time of 18 - 69 days was required. The minimum was recorded in *Phalaenopsis* on MS medium and the maximum was recorded in case of *Cattleya* on KC medium. Similarly, percentage of callusing was recorded in terms of success rate of explants producing callus and the degree of callusing in terms of amount of callus produced. Both these were studied by visual observation as precise qualitative estimation would disturb the *in vitro* process and also terminates the experiment itself.

To study the time required for the initiation of callus, shoot apices of all the experimental orchids were inoculated on to all the three basal media with 8 mg/l agar 15 g/l sucrose, 100 mg/l citric acid, 150 ml/l CW, 2 mg/l 2,4-D and varied Concentration (1-5 mg/l) of BAP, observations were made daily (see Tables 2-5).

Shoot tips of *Dendrobium Queen Sonia* (DQS) started producing callus, in VW medium with 5 mg/l BAP within 24 days of inoculation. They initiated callus after 42 days of inoculation, when the concentration of BAP was 1 mg/l in the same medium. In MS medium, the initiation of callus was comparatively slow. It occurred in 35 days with 5 mg/l BAP. Initiation of callus occurred in 41 days with 1 mg/l BAP. Initiation of
callus was slowest in KC medium it took nearly 62 days at 1 mg/l BAP concentration. But callusing started in about 39 days in the same medium with 3 mg/l BAP. Though the concentration of 2,4-D was constant (2 mg/l), the increase in the concentration of BAP from 1 mg/l to 5 mg/l in all the medium considerably hastened the speed of callus induction up to certain level. VM medium with 2 mg/l 2,4-D and 3 mg/l BAP was found to be the best combination for callus induction from shoot apices of DQS where callus was initiated within 21 days and only this callus was used for further studies (see Table 2).

Shoot apices of Dendrobium Emma white (DEW) also showed the similar response. But it was slightly faster in responding. In VWM with 5 mg/l BAP callusing started in about 23 days and it took 41 days to respond in the same medium with 1 mg/l BAP. In MS medium initiation of callusing started in 34 days with 5 mg/l BAP. Callusing initiated after 40 days with 1 mg/l BAP. KC medium with 1 mg/l BAP induced callusing in 65 days. But 1 mg/l BAP in the same induced callus in 47 days. VW medium supplemented with 3 mg/l BAP was found to be the best for callus induction from the shoot apices of DEM where callus initiation started in 20 days (see Table 3) and only this callus was used for further studies.

Shoot apex of Cattleya Naomi Kerns (CNK) showed faster callus induction in of MS medium as compared to VW and KC media. The callus
was induced within 26 days of inoculation in MSM with 5 mg l\textsuperscript{-1} BAP. In the same medium with 1 mg l\textsuperscript{-1} BAP callus induction occurred after 36 days. In KC medium callus induction was little slower. Shoot apex of *Cattleya* hybrid started producing callus after 39 days of inoculation in KC medium with 5 mg l\textsuperscript{-1} BAP and callus formation started in 48 days in the same medium with 1 mg l\textsuperscript{-1} BAP. In VW medium, callus induction began slowly. With 5 mg l\textsuperscript{-1} BAP callus formation started in 39 days and with 1 mg l\textsuperscript{-1} BAP it started after 46 days of inoculation.

MS medium with 4 mg l\textsuperscript{-1} BAP and 2 mg l\textsuperscript{-1} 2, 4-D was found to be the best combination for inducing callus formation the shoot apices of *Cattleya* hybrid (see Table 4) and only this callus was used for further studies.

Shoot apex of *Phalaenopsis Queen Emma* (PQE) initiated callus formation within 18 days in MS medium with 5 mg l\textsuperscript{-1} BAP. But callus formation started after 33 days in MS medium 1 mg l\textsuperscript{-1} BAP. In VW medium with 5 mg l\textsuperscript{-1} BAP shoot apex of *Phalaenopsis* hybrid started producing callus after 34 days and it took nearly 46 days in the same medium with 1 mg l\textsuperscript{-1} BAP. Shoot apex of *Phalaenopsis* hybrid initiated callus formation in KC medium supplemented with 1 mg l\textsuperscript{-1} BAP after 68 days of inoculation. Shortest time of only 18 days was taken by the shoot apices of *Phalaenopsis* hybrid to start producing callus in the MS medium.
with 5 mg l\(^{-1}\) BAP (see Table 5) and only this callus was used for further studies.

Callus started forming from the base of the short apices. Initially the callus was white in colour and slowly started turning green. As the callus started forming the explants turned brown or black in colour but sometimes they remained green and even produced very small leaves also. Though, initiation of callus was observed, in all the above combinations the nature and amount of callus formed varied considerably depending on the orchid and the medium used.

**PERCENT CALLUSING (PC) AND DEGREE OF CALLUSING (DC)**

Different explants were inoculated in all the three basal media with 8 g l\(^{-1}\) agar, 15 g l\(^{-1}\) sucrose, 100 mg l\(^{-1}\) citric acid, 150 ml\(^{-1}\) coconut water, 2 mg l\(^{-1}\) BAP as constant and varied concentrations of NAA (0.5 to 5 mg l\(^{-1}\)) and observations were made in 10 weeks old culture (see Table 9-20).

Callus was induced successfully in all explants used. It was found that leaf segments and internodal segments were comparatively difficult to induce callus. Meristem was the best explant but it was difficult to isolate it. Shoot apices and nodal buds were found to be the most viable explants as they showed good response and required simpler procedure. Root segments were also equally good for the induction of callus but they produced some unusual type of callus in certain concentrations and plants
obtained from the root explants are yet to show flowering whereas the plant obtained from all other explants have already flowered.

*Dendrobium* Queen Sonia in MS medium with 2 mg l⁻¹ BAP as constant and varied concentrations of NAA( see Table 9)

Meristem explants showed 61% callusing at 0.5 mg l⁻¹ NAA and 63% callusing at 5 mg l⁻¹ NAA. Maximum percentage of callusing (82%) was obtained at 2 mg l⁻¹ NAA. It showed 2.1 degree of callusing (DC) at 0.5 mg l⁻¹ NAA and 5.0 DC at 5 mg l⁻¹ NAA. DC increased with the increase in the concentration of NAA. Percent callusing increased initially but started declining beyond the optimum level of NAA concentration.

Shoot apex showed 55 and 48 percent callusing (PC) at 0.5 and 5 mg l⁻¹ concentrations of NAA respectively. 77 PC was recorded at 2 mg l⁻¹ NAA concentration. DC was 1.8 at 0.5 mg l⁻¹ NAA and 5.0 at 5 mg l⁻¹ NAA. Nodal buds showed 55 PC at 0.5 mg l⁻¹ NAA and 40 PC at 5 mg l⁻¹ NAA level. DC was 2.0 at 0.5 mg l⁻¹ NAA and 4.6 at 5 mg l⁻¹ NAA. Leaf segments showed low PC and DC. They showed no callusing at 0.5 mg l⁻¹ NAA where they remained green for about three weeks, they started turning brown and became black by 6 weeks. At mg l⁻¹ NAA leaf segments showed whitish callus in the margins. Leaf segments showed wrinkling and swelling after about 2 weeks. Callus started appearing o/n the margins in the fifth week. At this level (2 mg l⁻¹) of NAA 20% callusing was observed at 5 mg l⁻¹ NAA 5 PC was recorded.
Internodal segments showed 32 PC at 3 mg l⁻¹ NAA and 23% PC at 0.5 mg l⁻¹ NAA and 15 PC at 5 mg l⁻¹ NAA level. They recorded 1 DC at 0.5 mg l⁻¹ NAA and 2.4 DC at 5 mg l⁻¹ NAA concentration.

Root segments showed very good response at all levels of NAA. PC increased with the increase in NAA concentration, but it started declining from 3 mg l⁻¹ concentration onwards. It showed 60 and 53 PC at 0.5 and 5 mg l⁻¹ NAA levels respectively maximum PC (78) was observed at 2.0 mg l⁻¹ NAA level. DC showed a steady increase as the concentration of NAA increased. 2.0 DC was recorded at 0.5 mg l⁻¹ NAA level and 4.5 DC recorded at 5 mg l⁻¹ NAA.

_Dendrobium Queen Sonia_ in KC medium with 2 mg l⁻¹ BAP and varied concentration of NAA (see Table 11)

Meristem showed 70 PC at 2 mg l⁻¹ NAA and showed 59 PC both at 0.5 mg l⁻¹ and 5 mg l⁻¹ level. 2.0 DC was observed at 0.5 mg l⁻¹ NAA and 4.9 at mg l⁻¹ NAA concentration DC increased with the increase in the level of NAA concentration. Shoot apex showed a maximum of 69 PC at 2 mg l⁻¹ NAA concentration. It showed 50 and 48 PC at 0.5 mg l⁻¹ and 5 mg l⁻¹ NAA concentration respectively. 2.0 DC at 0.5 mg l⁻¹ NAA increased to 4.4 at 5 mg l⁻¹ NAA concentration. Both PC and DC were better in meristem explant as compared to shoot apex. Nodal buds showed 67 PC at 2 mg l⁻¹ NAA. 54 PC was recorded at 0.5 mg l⁻¹ NAA and 38 PC at 5 mg l⁻¹
NAA level. Overall DC was not as good as in meristem and shoot apex explant in this case. A maximum of 4 PC was recorded at 5.0 mg l⁻¹ NAA.

Leaf segments showed response to callus induction within the short range of 1 to 4 mg l⁻¹ NAA. 30 PC was recorded at 3 mg l⁻¹ NAA and 10 PC was recorded at 1 mg l⁻¹ NAA. DC was also very low and it was 1.5 at 2.0 mg l⁻¹ NAA level and DC decreased with both increase and decrease in the NAA level.

Internodal segments showed 20 PC at both 0.5 mg l⁻¹ and 4.0 mg l⁻¹ levels. A maximum of 30 PC was observed at 2.0 mg l⁻¹ concentration DC was ranged between 1 and 1.9. It increased as the concentration of NAA increased.

Root segments showed 65 PC at 3.0 mg l⁻¹ NAA and it was the optimum level. They showed 49 PC and 45 PC at 0.5 mg l⁻¹ and 5 mg l⁻¹ level of NAA respectively. DC was 1.5 at 0.5 mg l⁻¹ NAA and increased to 4.0 at 5 mg l⁻¹ NAA.

*Dendrobium* Queen Sonia in VW medium with mg l⁻¹ BAP as constant and varied concentrations of NAA (see Table10).

VWM was found to be best for all and, where maximum PC and DC was recorded in this medium.

91% of the meristems inoculated showed the formation of callus at 2mg l⁻¹ NAA level. By the end of sixth week yellowish masses of calli were formed at 2.0 mg l⁻¹ NAA level. AT 5 mg l⁻¹ NAA concentration.
68% of the meristems showed callusing and the remaining meristems dried up at 5 mg l\(^{-1}\) NAA level. DC was high (4.8) at 5 mg l\(^{-1}\) NAA level. 65 PC was observed and 2.2 DC was recorded, at 0.5 mg l\(^{-1}\) NAA.

Shoot apex showed 83 PC at 2 mg l\(^{-1}\) BAP. At 1 mg l\(^{-1}\) it showed 80 PC even meristem also showed a good rate of 89 PC at 1 mg l\(^{-1}\). NAA shoot apices showed 4.6 DC at 5.0 mg l\(^{-1}\) NAA and 2.5 DC at 0.5 mg l\(^{-1}\) NAA.

Nodal buds showed 75 and 76 PC at 1 mg l\(^{-1}\) and 2 mg l\(^{-1}\) NAA levels, respectively. At 3 mg l\(^{-1}\) and 0.5 mg l\(^{-1}\) levels of NAA they showed 49 and 66 PC respectively. DC however increased with the increase in the concentration of NAA.

At 0.5 mg l\(^{-1}\) NAA leaf segments showed no response. But showed a good response of 75 PC at 3 mg l\(^{-1}\) NAA and they showed 39 PC and 40 PC at 2 mg l\(^{-1}\) and 4 mg l\(^{-1}\) levels of NAA. A maximum of 2.0 DC was recorded at 2.0 mg l\(^{-1}\) NAA level.

Internodal segments showed a maximum of 39 PC at 2.0 mg l\(^{-1}\) and they showed a maximum of 2.0 DC at 5 mg l\(^{-1}\) NAA.

Root segments showed better response than the nodal buds and internodes. A maximum of 80 PC was observed 2 mg l\(^{-1}\) NAA and 79 PC at 1 mg l\(^{-1}\) NAA. PC was ranging between 60 to 50 with a maximum 80 in the concentrations between 0.5 mg l\(^{-1}\) to 5 mg l\(^{-1}\) of NAA. At 2mg l\(^{-1}\)
concentration 3.0 DC was observed but DC increased to 4.6 at 5 mg l⁻¹ NAA level.

*Dendrobium Emma White* in MS medium with 2 mg l⁻¹ BAP and varied concentration of NAA (see Table 12 fig. 39).

Meristem showed 90 PC at 1 mg l⁻¹ NAA level and showed 88 PC at 2 mg l⁻¹ in these levels of NAA. 2.5 and 3.0 DC were recorded. At 5 mg l⁻¹ NAA level 58 PC and 4.9 DC were recorded. At 0.5 mg l⁻¹ NAA level 60 PC and 2.1 DC were recorded.

Shoot apex showed 86 PC at 1.0 mg l⁻¹ NAA and 79 PC at 2.0 mg l⁻¹ NAA. At 0.5 mg l⁻¹ and 5 mg l⁻¹ NAA levels 58 and 50 PC were recorded. At 5 mg l⁻¹ NAA 5.0 DC was recorded at 2.2 DC was recorded at 0.5 mg l⁻¹ NAA concentration.

Nodal buds showed 72 PC at 1 mg l⁻¹ NAA and 67 PC at 2 mg l⁻¹ NAA. At 0.5 mg l⁻¹ and 5 mg l⁻¹ NAA concentrations they showed 49 and 45 PC. DC ranged between 1.9 and 3.8.

Internodal segments showed 68 PC at 2 mg l⁻¹ NAA and 58 PC at 1 mg l⁻¹ NAA. They showed 1.0 and 2.8 DCs at 0.5 mg l⁻¹ and 5 mg l⁻¹ NAA concentrations respectively.

It was difficult to induce callus in leaf segments. There was not much of a difference for induction of callus is concerned in basal, middle and apical regions of the leaf. Leaf segments showed very slow callus formation. Callus formed from the cut regions of the leaf segments. It
appeared as white growth first, turning yellow gradually. At 0.5 mg l\(^{-1}\) NAA the leaf segments turned yellow first and then turned brown and there was no callus formation, and all segments died. At 2.0 mg l\(^{-1}\) about 48% of leaf segments showed callus formation within six weeks, whereas the others wrinkled and remained green for another two weeks and then turned brown and died. At 2 mg l\(^{-1}\) NAA level 3.1 DC was recorded.

Root segments showed response to callus induction at all levels of NAA studied. At 3 mg l\(^{-1}\) NAA level they showed 70 PC. PC ranged between 45 to 50. DC was maximum (4.5) at 5 mg l\(^{-1}\) NAA concentration.

*Dendrobium* Emma White in KC medium with 2 mg l\(^{-1}\) BAP as constant and varied concentration of NAA (see Table, 14 fig. 29).

Meristem showed 86 PC in 2 mg l\(^{-1}\) NAA concentration. It showed 60 PC both at 0.5 mg l\(^{-1}\) and 5 mg l\(^{-1}\) NAA concentrations. It showed 4.8 DC at 5 mg l\(^{-1}\) NAA concentration. Shoot apex showed 84 PC at 2 mg l\(^{-1}\) NAA concentration. It recorded 60 PC 62 PC at 0.5 and 5 mg l\(^{-1}\) NAA levels. It showed 4.6 DC at 5 mg l\(^{-1}\) NAA levels respectively. It showed 4.6 DC at 5 mg l\(^{-1}\) NAA concentration. But at 0.5 mg l\(^{-1}\) level it showed 1.6 DC.

Nodal buds showed 72 PC at 2 mg l\(^{-1}\) NAA level and showed 48 and 49 PC at 0.5 mg l\(^{-1}\) and 5 mg l\(^{-1}\) NAA levels respectively. They showed 3.9 DC at 5 mg l\(^{-1}\) NAA level. Leaf segments did not show any response at 0.5 mg l\(^{-1}\) and 1 mg l\(^{-1}\) NAA levels. At 2.0 mg l\(^{-1}\) level leaf segments showed 22
PC. DC ranged between 0.5 at 5 mg l\(^{-1}\) NAA level to 1.2 at 2 mg l\(^{-1}\) level. Internodal segments showed a maximum of PC 23 of PC at 2 mg l\(^{-1}\) level was even lower at 0.5 mg l\(^{-1}\) and 5 mg l\(^{-1}\), where it was 10 and 13 respectively. Root segments showed 75 PC at 2 mg l\(^{-1}\) NAA concentration and 4.5 DC at 5 mg l\(^{-1}\) NAA concentration.

*Dendrobium* Emma White in VW medium with 2 mg l\(^{-1}\) BAP as constant and varied concentrations of NAA (see Table 13, fig. 31).

VWM was found to be the best medium for this hybrid. Meristem followed by shoot apex were the best explants.

Meristem showed 92 PC at 2 mg l\(^{-1}\) NAA. It showed 65 PC at 0.5 mg l\(^{-1}\) level and 66 PC at 5 mg l\(^{-1}\) NAA concentrations. This explant showed 5.0 DC at 5 mg l\(^{-1}\) NAA concentration and 1 DC at 0.5 mg l\(^{-1}\) NAA concentration and at 2 mg l\(^{-1}\) NAA level it showed 3.2 DC.

Shoot apex showed 86 PC at 2 mg l\(^{-1}\) NAA level at this level it showed 2.5 DC. It showed 45 PC at 5 mg l\(^{-1}\) NAA and 61 PC at 0.5 mg l\(^{-1}\) NAA. DC was maximum at 5 mg l\(^{-1}\) NAA where it recorded 4.5 DC.

Nodal buds showed good response in the above medium. They started producing callus by the end of fifth week after inoculation. Buds in contact with medium produced callus whereas the cut stem region along with which the buds were inoculated remained green. Only the cut terminal end turned black. Buds showed 78 PC at 2 mg l\(^{-1}\) NAA level and 69 PC was recorded at 0.5 mg l\(^{-1}\) NAA level. PC was 30 at 5 mg l\(^{-1}\) NAA.
level. DC ranged between 1.0 at 0.5 mg l⁻¹ NAA and 4.4 at 5mg l⁻¹ NAA levels.

Leaf segments did not respond at lower levels of NAA concentration. At 3 mg l⁻¹ NAA a maximum of 28 PC was observed and at 4 mg l⁻¹ NAA concentration 15 PC was observed at this level 3 DC was recorded.

Internodal segments showed 38 PC at 2.0 mg l⁻¹ NAA concentration at this level of NAA they showed 2.2 DC. This explant showed a maximum of 3.3 DC at 5 mg l⁻¹ NAA concentration.

Root segments showed 80 PC and 79 PC at 1mg l⁻¹ and 2 mg l⁻¹ NAA levels respectively. DC was found to be 0.5 at 0.5 mg l⁻¹ NAA level which increased to 4.8 at 5 mg l⁻¹ NAA level.

Cattleya Naomi Kerns in VW medium with 2 mg l⁻¹ BAP and varied concentrations of NAA (see Table 16)

In case of meristem explants 89 PC was recorded at 1.0 mg l⁻¹ NAA level 0.5 mg l⁻¹ NAA and 5 mg l⁻¹ NAA levels recorded 58 and 48 PC. DC increased from 1.9 at 0.5 mg l⁻¹ NAA level to 5.0 at 5 mg l⁻¹ NAA level.

Shoot apex showed 86 PC at 1.0 mg l⁻¹ NAA and it showed 56 and 45 PC at 05 mg l⁻¹ NAA and 5 mg l⁻¹ NAA levels respectively and 5.0 DC observed at 5 mg l⁻¹ NAA concentration.
Nodal buds showed 79% callusing at 1 mg l⁻¹ NAA. PC decreased as with increase in the NAA concentration above this level. At 1 mg l⁻¹ NAA level they showed 2.3 DC, it increased to 3.9 at 5 mg l⁻¹ NAA level.

Leaf segments showed callusing at all concentrations range of 1 mg l⁻¹ to 5 mg l⁻¹. Leaf segments showed swelling and wrinkling within a week’s time. They remained green for nearly two weeks. Then they turned yellow-green and subsequently produced whitish yellow callus. Leaf bases gave better results compared to laminar and apical regions of the leaf. At 2.0 mg l⁻¹ NAA leaf segments showed 38 PC and at 1 mg l⁻¹ NAA 52 PC was observed, but DC ranged between 1.0 to 1.8. It was maximum (2.5) at 5.0 mg l⁻¹ NAA level.

Internodal segments showed 49 PC at 1 mg l⁻¹ level and at this level they showed 2.2 DC. Root segments however, showed better response as compared to leaf segments, internodal segments and nodal buds. They showed 80 PC at 1.0 mg l⁻¹ NAA which got decreased as the NAA level increased. At 1.0 mg l⁻¹ NAA concentration 2.0 DC recorded, which increased to 3.9 at 5 mg l⁻¹ NAA.

*Cattleya Naomi Kerns* in MS medium with 2 mg l⁻¹ BAP and varied concentration of NAA (see Table 15, figs.52, 57 and 60).

For callus induction in this hybrid, MS was the best medium. Most of the explants showed good callusing at 1 mg l⁻¹ NAA level. Meristem showed 90 PC at 1 mg l⁻¹ NAA. It showed 58 PC and 50 PC at 0.5 mg l⁻¹
NAA and 5 mg l\(^{-1}\) NAA concentrations respectively and 4.6 DC was observed at 5 mg l\(^{-1}\) NAA.

Shoot apex showed 88 PC at 1 mg l\(^{-1}\) NAA and it showed 4.9 DC at 5 mg l\(^{-1}\) NAA.

Nodal buds showed 86 PC at 1 mg l\(^{-1}\) NAA. At 5 mg l\(^{-1}\) NAA they showed 44 PC. A maximum of 4.2 DC was observed at 5 mg l\(^{-1}\) NAA.

Leaf segments did not show any response at 0.5 mg l\(^{-1}\) NAA 40 PC was observed at 1 mg l\(^{-1}\) NAA and 2.5 DC was recorded at 5 mg l\(^{-1}\) NAA.

Internodal segments showed 77 PC at 1 mg l\(^{-1}\) NAA. They showed 40 PC both at 0.5 mg l\(^{-1}\) NAA and 5.0 mg l\(^{-1}\) NAA levels. A maximum of 3.0 DC was observed at 5 mg l\(^{-1}\) NAA level.

Root segments showed 79 PC at 2 mg l\(^{-1}\) NAA, and at this level they showed 2.6 DC. But at 5 mg l\(^{-1}\) NAA concentration they showed 4.0 DC.

*Cattleya Naomi Kerns* in KC medium with 2 mg l\(^{-1}\) BAP as constant and varied concentration of NAA (see Table 17)

Meristem explants showed 73 PC at 2 mg l\(^{-1}\) NAA level at this level 3.9 DC was observed. PC decreased with the increase in the NAA concentration. At 5 mg l\(^{-1}\) NAA level 4.5 DC was observed.

Shoot apex showed 69 PC at 2 mg l\(^{-1}\) NAA and at this level it showed 2.1 DC. 4.0 DC was observed at 5 mg l\(^{-1}\) NAA level.

Nodal buds showed 68 PC and 1.6 DC at 2.0 mg l\(^{-1}\) NAA. At 5 mg l\(^{-1}\) NAA they showed 33 PC and 2.6 DC.
Leaf segments did not show any response both at 0.5 mg l\(^{-1}\) NAA and 5 mg l\(^{-1}\) NAA. They showed 25 PC and 1.0 DC at 1 mg l\(^{-1}\) NAA concentration and it was the best response obtained.

Internodal segments showed 44 PC and 1.8 DC at 2.0 mg l\(^{-1}\) NAA and at 5 mg l\(^{-1}\) NAA they showed 25 PC and 2.2 DC.

Root segments showed 60 PC and 3.0 DC at 2.0 mg l\(^{-1}\) NAA at 5 mg l\(^{-1}\) NAA concentration they showed 30 PC and 3.8 DC.

*Phalaenopsis Queen Emma* in MS Medium with 2 mg l\(^{-1}\) BAP and varied concentration of NAA (see Table 18, figs. 77, 82 and 85).

In this hybrid to obtain most of the explants inflorescence stalk was used. For example instead of shoot apex, inflorescence apex, instead of stem nodal buds inflorescence nodal buds and instead of internodal segments of stem, internodal segments of inflorescence stalk were used. All these segments showed good response.

Meristem gave best results. It showed 90 PC at 2.0 mg l\(^{-1}\) NAA concentration. The callus which was initially whitish turned green after three weeks of callus formation. Meristem showed 90 PC at 2 mg l\(^{-1}\) NAA and 4.9 DC at 5.0 mg l\(^{-1}\) NAA level. Inflorescence apex showed 80, 85 and 90 PC at 0.5 mg l\(^{-1}\), 1.0 mg l\(^{-1}\) and 2 mg l\(^{-1}\) NAA levels respectively. But maximum DC of 5.0 was recorded at 5 mg l\(^{-1}\) NAA.
Nodal buds showed 77 and 78 PC at 1.0 and 2.0 mg l⁻¹ NAA levels. But DC was maximum, 4.8 at 5 mg l⁻¹ NAA level.

Leaf segments obtained from young plants showed good response. Basal, as well as the apical regions showed response for callusing. In leaf segments 50 PC was observed at 2.0 mg l⁻¹ NAA and 2.8 DC, which was the maximum for this explant was observed at 5 mg l⁻¹ NAA. At this concentration PLBs and plantlets were directly produced from the cut regions of the leaf segments. This occurred without the appearance of callus.

Internodal segments showed 79 PC and 2.7 DC at 2.0 mg l⁻¹ NAA. They showed 42 PC and 3.2 DC at 5 mg l⁻¹ NAA levels.

Root segments showed 76 and 78 PC at 1.0 mg l⁻¹ and 2.0 mg l⁻¹ NAA levels respectively. They showed 41 PC and 4.2 DC at 5 mg l⁻¹ NAA level.

*Phalaenopsis* Queen Emma in KC medium with 2 mg BAP as constant and varied concentrations of NAA (see Table 19, figs. 80 and 81).

Meristem showed 75 PC at 2 mg l⁻¹ NAA level, and 2.9 DC at this level of NAA. It showed 60 PC and 4.8 DC at 5 mg l⁻¹ NAA concentration. Inflorescence apex showed 70 PC and 2.8 DC at 2 mg l⁻¹ NAA. It showed 50 PC and 4.9 DC at 5 mg l⁻¹ NAA concentration.

Nodal buds showed 72 PC and 3.0 DC at 2 mg l⁻¹ NAA and 48 PC and 4.8 DC at 5 mg l⁻¹ NAA level.
Leaf segments showed 40 PC and 2.0 DC at 2 mg l\(^{-1}\) NAA. At 5 mg l\(^{-1}\) NAA they showed 5 PC and 1.0 DC. No response was noted at 0.5 and 1 mg l\(^{-1}\) NAA.

Internodal segments showed no response at 0.5 mg l\(^{-1}\) NAA. At 2.0 mg l\(^{-1}\) NAA they showed 51 PC and 1.8 DC and at 5 mg l\(^{-1}\) NAA 15 PC and 2.2 DC were observed.

Root segments showed no response at 0.5 mg l\(^{-1}\) NAA. But at 2.0 mg l\(^{-1}\) NAA they showed 68 PC and 2.2 DC. DC was maximum, 3.9 at 5.0 mg l\(^{-1}\) NAA concentration.

*Phalaenopsis Queen Emma* in VW medium with 2 mg l\(^{-1}\) BAP and varied concentration of NAA (see Table 20, figs. 78, 79, and 83).

VWM was found to be the best medium for callus induction in almost all explants of this hybrid.

Meristem showed 90 PC at 1 mg l\(^{-1}\) NAA and at this level of NAA 2.5 DC was recorded. At 5 mg l\(^{-1}\) NAA level 45 PC and 5.0 DC was observed.

Inflorescence apex showed 90 PC at 2.0 mg l\(^{-1}\) and 85 PC at 1.0 mg l\(^{-1}\) NAA. It showed 4.5 DC at 4.0 mg l\(^{-1}\) NAA and 5.0 DC at 5 mg l\(^{-1}\) NAA concentrations.

Nodal buds showed 79 PC at 1.0 mg l\(^{-1}\) NAA and 82 PC at 2 mg l\(^{-1}\) NAA. They showed 3.9 DC at 4.0 mg l\(^{-1}\) NAA and 4.5 DC at 5.0 mg l\(^{-1}\) NAA.
Leaf segments showed response at all levels of NAA tried. PC was lowest (20) at 5.0 mg l\(^{-1}\) and highest (50) at 2.0 mg l\(^{-1}\) NAA. Maximum DC was recorded (2.5) at 5 mg l\(^{-1}\) NAA concentration.

Internodal segments showed 60 PC and 2.8 DC at 3.0 mg l\(^{-1}\) NAA and 42 PC and 2.9 DC at 5.0 mg l\(^{-1}\) NAA. Root segments showed 69 PC and 3.0 DC at 2.0 mg l\(^{-1}\) NAA and 39 PC and 4.1 DC at 5 mg l\(^{-1}\) NAA concentration.

**MULTIPLICATION OF CALLUS**

The degree of multiplication of callus (DM) was observed visually and was categorized under five observable visual stages, as detailed in methods. These stages represent the relative quantities of callus growth from the callus segments subcultured. Callus obtained from the shoot tip was used in this study (see Table 21).

Callus was segmented into small pieces and inoculated on all the three basal media with 30 mg l\(^{-1}\) sucrose, 8g l\(^{-1}\) agar, 100 mg l\(^{-1}\) ascorbic acid and varied concentrations of NAA and BAP.

*Dendrobium Queen Sonia* showed 5 DM at 2 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP in MS medium. At the same level of growth regulators in VW medium it showed 4.6 DM and in KC medium it showed 3.8 DM. DM increased with the increase in NAA and BAP levels but after the above mentioned levels it again started decreasing.
Dendrobium Emma White also showed the similar results. At 2 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP at showed 5 DM, 4.8 DM and 4.0 DM is MS, VW and KC media respectively.

Cattleya hybrid showed a maximum of 5 DM at 2 mg l\(^{-1}\) NAA and 3 mg l\(^{-1}\) BAP in MS medium at this level it showed 4.2 DM and 3.8 in VW and KC media respectively. It showed 4 DM at 1 mg l\(^{-1}\) NAA and 3 mg l\(^{-1}\) BAP in MS medium.

Phalaenopsis hybrid showed 5 DM, 4 DM and 3.3 DM at 3 mg l\(^{-1}\) and 2 mg l\(^{-1}\) BAP in MS, VW and KC media respectively. In MSM at 1 mg l\(^{-1}\) NAA and 3 mg l\(^{-1}\) BAP, it rewarded 4.1 DM and at 3 mg l\(^{-1}\) each of BAP and NAA in MSM is showed 4.4 DM.

Requirement of NAA and BAP for callus multiplication was found to be low in Dendrobium hybrids, moderate in Cattleya and high in Phalaenopsis. PLBs and occasionally plantlets were also produced in the above treatments.

**REGENERATION OF PLBS AND PLANTELETS**

For this study different explants and callus segments obtained from the earlier stages were inoculated on all the three selected media with varied concentration of growth regulators (see Tables 22-41, figs. 5-7, 32, 33, 53, 54, 56, 58, 59, 86, 90 and 91-94).
As in earlier cases, here also visual, non parametric. Observations were made at regular intervals for 10 weeks and recorded as in Tables mentioned. The purpose of this study was to identify the best medium suited for the individual orchid selected for study. Explants from shoot apices of all orchids yielded good results. VW medium was found to be suitable for *Dendrobium* hybrids and MS medium was better for *Cattleya* and *Phalaenopsis*. Hence these combinations were continued further in this study. Performance in terms of weight of the callus produced, percentage of explants survived, percentage of explants producing callus and percentage of PLB formation were studied.

A similar experiment was also conducted with callus segments by growing them on selected media. In addition to the parameters of percentage of survival and percentage of PLB formation, colour of PLBs and number of plantelets derived from each segment cultured have also been studied. For the purposes of above studies explants and callus segments were inoculated on all the three basal media with 20 g l\(^{-1}\) sucrose, 100 mg l\(^{-1}\) ascorbic acid, 100 mg l\(^{-1}\) citric acid, 1 mg l\(^{-1}\) BAP and varied concentration of NAA.

*Dendrobium Queen Sonia* showed highest degree of PLB formation in VW medium while it was moderate in MS medium. In KC medium the degree of PLB formation was least. In all the cases, increase in the concentration of NAA decreased the degree of PLB formation beyond 1
mg l\(^{-1}\) level and best results were obtained at 0.5 mg l\(^{-1}\) NAA. Of all the explants used shoot apex, inflorescence apex, nodal buds and callus segments showed high degree of PLB formation. Root segments showed least degree of PLB formation and they required higher levels of NAA.

*Dendrobium* Emma White showed high degree of PLB formation in VW medium. In MS medium it showed moderate response. Least degree response was observed in KC medium 0.5 mg l\(^{-1}\) NAA level was found to be most suitable for shoot apex. Inflorescence apex, callus segments and leaf segments responded well both at 0.5 mg l\(^{-1}\) and 1 mg l\(^{-1}\) NAA levels whereas, the root segments showed best response at 2 mg l\(^{-1}\) NAA level. Degree of PLB formation decreased in all explants as the concentration of NAA increased and at 5 mg l\(^{-1}\) NAA level the degree of PLB formation was almost nil.

In case of *Cattleya* hybrid the degree of PLB formation was best in MSM. It was moderate in VW medium. In KC medium degree of PLB formation was found to be least. The response was very good at 1 mg l\(^{-1}\) NAA level. All explants except root segments showed best response at this level of NAA. Moderate response was observed at 0.5 mg l\(^{-1}\) NAA level. Root explants showed best response at 2 mg l\(^{-1}\) NAA level. Though the degree of response varied, callus segments showed PLB formation at almost all levels (0.5 - 5 mg l\(^{-1}\)) of NAA whereas other explants failed to show PLB formation at higher levels of NAA.
In *Phalaenopsis* Queen Emma best degree of PLB formation was observed in MSM. The response was moderate in VW medium. In KC medium it was least *Phalaenopsis* hybrid showed the requirement of high NAA concentration. All explants except root segments showed good degree of PLB formation at 5 mg l\(^{-1}\) NAA level. The response was moderate at 4 mg l\(^{-1}\) NAA. Degree of PLB formation was least at lower NAA concentrations. Root segments however, showed good response at 3 mg l\(^{-1}\) NAA level. In MS medium root segments showed equal degree of PLB formation at 3 mg l\(^{-1}\), 4 mg l\(^{-1}\) and 5 mg l\(^{-1}\) concentration of NAA. White callus appeared from the explants at the point of contact with medium. Gradually the white callus turned green at some places and started producing PLBs.

To study the percent survival of explants, wet weight of the callus produced and percent of PLB formation, shoot apices of *Dendrobium* and *Cattleya* hybrids and inflorescence apex of *Phalaenopsis* hybrid were inoculated as MS medium, VW medium or KC medium with 15 g l\(^{-1}\) sucrose 8.5 g l\(^{-1}\) agar, 100 mg l\(^{-1}\) citric acid, 100 mg l\(^{-1}\) ascorbic acid, and varied concentrations of BAP and 2, 4-D.

*Dendrobium* Queen Sonia shoot apices were inoculated on VW medium. The percent survival was 95 at 0.1 mg l\(^{-1}\) 2, 4-D and 0.1 mg l\(^{-1}\) BAP level. The same result was obtained even at 1 mg l\(^{-1}\) 2, 4-D and 1 mg l\(^{-1}\) BAP. Percent survival decreased with the increase in the concentration
of both 2, 4-D and BAP. At higher levels of growth regulators the percent survival was low. On the contrary the wet weight of the callus formed from each shoot apex explant increased with the increase in the concentration of both 2, 4-D and BAP. Wet weight was found to be maximum (482 mg) at 2 mg l⁻¹ BAP and 5 mg l⁻¹ 2, 4-D level.

Percent PLB formation showed decrease with the increase in the concentration of 2, 4-D and BAP. Maximum PLB formation (70%) was observed at 1 mg l⁻¹ BAP and 1 mg l⁻¹ 2, 4-D. No PLB formation was observed when 2, 4-D was used at higher levels than 4 mg l⁻¹.

Dendrobium Emma White shoot apices were inoculated onto VW medium and they showed decrease in the percent survival with the increase in the 2, 4-D level. At 0.1 mg l⁻¹ 2, 4-D and 0.1 mg l⁻¹ BAP level 90 percent survival was recorded. At 1 mg l⁻¹ BAP and 0.1 mg l⁻¹ 2, 4-D maximum percent survival (95) was noticed. A minimum of 0.5 percent survival was recorded at 0.1 mg l⁻¹ BAP and 5 mg l⁻¹ 2, 4-D. 25 percent survival was recorded 2 mg l⁻¹ BAP and 0.1 mg l⁻¹ 2, 4-D level. Wet weight of the callus increased with the increase in the concentration of BAP and 2, 4-D. Minimum wet weight (292 mg) of callus was recorded at 0.1 mg l⁻¹ BAP and 0.1 mg l⁻¹ 2, 4-D. Maximum wet weight (542 mg) of callus was recorded at 2 mg l⁻¹ BAP and 5 mg l⁻¹ 2, 4-D.

Percent of PLB formation was decreased with the increase in the concentration of 2, 4-D and BAP. 65 percent PLB formation was recorded
at 0.1 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D level. At 1 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D percent PLB formation was 60. There was no PLB formation at higher levels of 2, 4-D.

*Cattleya Naomi Kerns* shoot apices were cultured on MS medium. They showed 100 percent survival at 0.1 mg l\(^{-1}\) 2, 4-D and 0.1 mg l\(^{-1}\) BAP level. But percent survival decreased with the increase in the concentration of BAP and 2, 4-D. Percent survival was 90 at 1 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D. At 5 mg l\(^{-1}\) 2, 4-D and 0.1 mg l\(^{-1}\) BAP, the percent survival was as low as 5.

Wet weight of the callus formed increased with the increase in the concentration of BAP and 2, 4-D. Lowest wet weight (310 mg) was recorded at 1 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D level. The highest wet weight (553 mg) was observed at 2 mg l\(^{-1}\) BAP and 5 mg l\(^{-1}\) 2, 4-D concentration.

Percent PLB formation was maximum (90) at 1 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D level, even at 2 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D 90 percent PLB formation was recorded. But at 2 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) 2, 4-D PLB formation was 45 percent. As there was increase in the concentration of 2, 4-D PLB formation decreased and no PLB formation recorded at 3, 4 and 5 mg l\(^{-1}\) level of 2, 4-D.

*Phalaenopsis Queen Emma* inflorescence apices were cultured on MS medium. They showed 90 percent survival at 0.1 mg l\(^{-1}\) BAP and 1 mg
l^1 2, 4-D. At 0.1 mg l^1 BAP and 0.1 mg l^1 2, 4-D they showed 85 percent survival. But as the concentration of 2, 4-D was increased beyond 1 mg l^1 i.e., at 2, 3, 4 & 5 mg l^1 the percent survival was 75, 50 35 and 15 respectively. At 2 mg l^1 BAP and 0.1 mg l^1 2, 4-D 80 percent survival was recorded. Percentage of survival gradually decreased as there was further increase in the concentration of 2, 4-D. Wet weight of the callus was increased with the increase in the concentration of 2, 4-D. At 0.1 mg l^1 2, 4-D and 0.1 mg l^1 BAP level 250 mg wet weight was recorded and at 0.1 mg l^1 BAP and 5 mg l^1 2, 4-D the wet weight was 306 mg. Wet weight was maximum at 2 mg l^1 BAP and 5 mg l^1. At this level 458 mg callus was formed.

Percent PLB formation was maximum (80) at 2 mg l^1 BAP and 0.1 mg 2, 4-D. It was 60 at 1 mg l^1 BAP and 2 mg l^1 2, 4-D level and 45 percent at 0.1 mg BAP mg l^1 and 2 mg l^1 2, 4-D. But at higher concentrations of 2, 4-D percent PLB formation was low and there was no PLB formation at 1 mg l^1 BAP and 5 mg l^1 2, 4-D level.

To study the effects of NAA and BAP on percent survival, percent PLB formation colour of PLBs and number plantlets formed directly from callus segments of all the orchid hybrids were inoculated as different (MS, VW or KC) media with 8.5 gms l^1 agar, 20 gms l^1 sucrose and varied concentration of BAP and NAA.
**Dendrobium** Queen Sonia callus segments were inoculated on VWM. Percent survival was a maximum (100) at 1 mg l⁻¹ NAA and 1 mg l⁻¹ BAP. At 1 mg l⁻¹ NAA and 5 mg l⁻¹ BAP concentration, 48 percent survival was recorded. At 5 mg l⁻¹ NAA and 1 mg l⁻¹ BAP 22 percent survival was observed and at 5 mg l⁻¹ NAA and 15 mg l⁻¹ BAP percent survival was zero. Minimum percent of survival (6) was recorded at 10 mg l⁻¹ NAA and 5 mg l⁻¹ BAP and 15 mg l⁻¹ NAA and 1 mg l⁻¹ BAP. No survival of callus segments recorded, when the concentration of BAP and NAA was above 10 mg l⁻¹.

Percent PLB formation also decreased with the increase in the concentration of NAA and BAP. 70 percent PLB formation was recorded at 1 mg l⁻¹ NAA and 1 mg l⁻¹ BAP. At 1 mg l⁻¹ NAA and 5 mg l⁻¹, 20 percent PLB formation was recorded. At 5 mg l⁻¹ NAA and 1 mg l⁻¹ BAP 18 percent PLB formation was observed. Minimum percent PLB formation was observed at 10 mg l⁻¹ NAA and 1 mg l⁻¹ BAP, where 4 percent PLB formation was recorded. There was no PLB formation when the concentration of NAA was higher than 5 mg l⁻¹. Colour of the PLBs was found to be yellow or pale yellow or pale green or green. PLB formed with lower concentration of growth regulators gave rise to plants more often. If the concentrations were increased, PLBs were pale green and pale yellow. PLBs under the influence of high concentration were yellow in colour. Pale yellow and yellow PLBs turned green gradually later on these
observations were made after 12 weeks of culture percent of yellow PLBs turning green reduced with the increase in the concentration of growth regulators.

At 1 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP level about fire plantlets were formed directly from the callus segments. Rate of plantlet formation was also decreased with the increase in the concentration growth regulators. Under the influence of high concentrations no plantlets were produced. When the PLBs produced were subcultured on VW (growth medium) green and pale green PLBs always formed plantlets. Pale yellow PLBs showed slow growth. Yellow PLBs either showed no growth or died.

*Dendrobium* Emma White showed similar results as those of the other *Dendrobium* hybrid, when its callus segments were inoculated onto VW medium. 98 percent survival was recorded at 1 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP level. At 1 mg l\(^{-1}\) NAA and 5 mg l\(^{-1}\) BAP level 52 percent survival was recorded. At 5 mg l\(^{-1}\) NAA and 10 mg l\(^{-1}\) BAP 10 percent survival was observed. At 1.5 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP 2 percent survival was observed. Survival percentage decreased with the increase in the concentration of NAA and BAP. Percent PLB formation was 68 at 1 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP level. Percent PLB formation decreased with the increase in the concentration of growth regulators. PLBs were not formed at higher levels of growth regulators. PLBs were green when the concentration of growth regulators was lower. As the concentration
increased PLBs turned pale green or yellow or pale yellow. When these PLBs were subcultured on VW medium all green PLBs and many of the pale green PLB formed plantlets yellow and pale yellow PLBs showed no further growth or died.

*Cattleya* Naomi Kerns callus segments were cultured on MS medium. At 1 mg l⁻¹ NAA and 1 mg l⁻¹ BAP, the percent survival was 100, and it was 62 at 1 mg l⁻¹ NAA and 5 mg l⁻¹ BAP. Though the percent survival decreased with the increase in the concentration of BAP and NAA, the mortality rate was less as compared to *Dendrobium* hybrids. Percent survival was zero at 5 mg l⁻¹ NAA and 15 mg l⁻¹ BAP and 15 mg l⁻¹ NAA and 15 mg l⁻¹ BAP levels. Percent PLB formation was 90 at 1 mg l⁻¹ NAA and 1 mg l⁻¹ BAP and it was 48 at 1 mg l⁻¹ NAA and 5 mg l⁻¹ BAP. At 5 mg l⁻¹ NAA and 1 mg l⁻¹ BAP PLB formation was 32 percent. There was no PLB formation at higher levels of growth regulators. PLBs produced under the influence of lower concentration of growth regulators were green and pale green in colour. They were yellow and pale yellow when produced using higher concentration of growth regulators. Green and pale green PLBs produced plantlets when subcultured on MS medium. Yellow and pale yellow PLBs either showed slow growth or died.

*Phalaenopsis* Queen Emma callus segments were cultured on MS medium. At 5 mg l⁻¹ NAA and 5 mg l⁻¹ BAP level a maximum of 80 percent survival was recorded. At 5 mg l⁻¹ NAA and 1 mg l⁻¹ BAP 64
percent survival was recorded. At both there levels green PLBs formed which eventually developed into plantlets on subculturing in MS medium. At 1 mg l\(^{-1}\) NAA and 1 mg l\(^{-1}\) BAP 60 percent survival was recorded where 20 percent PLB formation was seen the PLBs were pale green which developed into plantlets directly. In this hybrid, green PLBs were developed when the concentration of growth regulators was higher. All green and pale green PLB produced plantlets directly and on subculturing as well. At 10 mg l\(^{-1}\) NAA level percent survival and percent PLB formation was ranging between 2 to 6. At 15 mg l\(^{-1}\) NAA level percent survival was zero.

**PLANTLET GROWTH AND FORMATION OF ROOTS**

A plantlet for the purposes of this study is defined as a differentiated PLB with at least one pair of leaves and several leaf primordia and with or without roots. Such plantlets measuring about 0.5 cm, were inoculated on different media with varied concentration of growth regulators (see Tables 42-62, figs. 10-14, 16-20, 36, 40, 47, 63-72, 96-99, 101, 102, 104, and 106).

Growth was non parametrically studied with the intention to select the suitable medium for growth (see Table 39 - 50) studies were also made to examine the effect of modified rooting media containing banana pulp and growth regulators (see Table 51). Here also non-parametric measurements were used.
After identifying VW medium for Dendrobiums and MS medium for Cattleya and Phalaenopsis as the best media for plantlet growth. Experiments were conducted to identify the most suitable growth regulator combination. Parameters like length of the plantlet, numbers of leaves and roots produced were recorded at the end of tenth week of culture.

Effects of NAA and BAP and nutrient media on the growth of the plantlets was studied by inoculating plantlets of all the orchid hybrids on VW, MS and KC basal media with 8.5gl⁻¹ agar 150 mg l⁻¹ coconut water, and 20 gm l⁻¹ sucrose (See Table 39-50).

Dendrobium Queen Sonia plantlets showed best growth in VW medium, moderate growth was observed in MS medium and slow a growth in KC medium. Growth increased with the increase in the concentration of NAA and with increase in the concentration of BAP growth reduced but showed formation of side shoots. When the concentration of NAA was higher than 5 mg l⁻¹, unusual growth was seen. At 10 mg l⁻¹ NAA level plants were unusually tall with fewer narrow leaves and long internodes (see Tables 39-41) and at 1 mg l⁻¹ BAP and 5 mg l⁻¹ NAA in VW medium healthy growth of plants with the formation of side shoots was observed. No growth was seen when the concentration of BAP and NAA was 10 mg l⁻¹.

Dendrobium Emma White plantlets showed very good growth in VW medium, moderate growth was recorded in MS medium, and slower growth was recorded in KC medium. BAP was found to be useful for
growth only in lower concentrations. In the absence of BAP at 5 mg l⁻¹ NAA good growth of plantlets was recorded in both VW and MS media. But at 7 mg l⁻¹ and 10 mg l⁻¹ NAA concentrations unusual growth of plantlets was observed. Increase in the BAP decreased the growth of plants associated with formation of side shoots was recorded. No growth was observed when the concentration of BAP and NAA was 10 mg l⁻¹ each NAA and BAP were inhibitory at higher concentrations (see also figs.45 and 46 for variations).

*Cattleya* Naomi Kerns plantlets showed very good growth in MS medium. In VW medium the growth was good. Slower growth was observed in KC medium. In MS medium, at 3 and 5 mg l⁻¹ NAA concentrations excellent growth was noticed. At 1, 7 and 10 mg l⁻¹ NAA levels growth was moderate. At 1 mg l⁻¹ BAP was 5 mg l⁻¹ NAA also very good growth was recorded. At 3 mg l⁻¹ BAP and 5 mg l⁻¹ NAA there was good growth and good formation of side shoots. There was no growth in the combination of high levels of NAA and BAP. Almost similar responses were elicited with both the growth regulators in VW and KC media (see Table 45-47).

*Phalaenopsis* Queen Emma plantlets showed the requirement of MS medium for good and healthy growth. In VW medium moderate growth was observed KC medium induced slower growth. In MS medium excellent growth was recorded at 1 mg l⁻¹ BAP and 7 and 10 mg l⁻¹ NAA.
At 3 mg l\(^{-1}\) BAP and 7 mg l\(^{-1}\) NAA lend there was good healthy growth was recorded besides side shoot formation. BAP alone in the absence of NAA did not induce growth but at higher levels it induced side shoot formation (see Tables 48-50).

Effect of banana pulp (BP) was studied on the growth and rooting of the plantlets of all the orchid hybrids in different selected basal media with 8.5 gl\(^{-1}\) agar 1mg l\(^{-1}\) BAP, 5 mg l\(^{-1}\) NAA, 500 mgl\(^{-1}\) activated charcoal and 20 gm l\(^{-1}\) sucrose 150 ml\(^{-1}\) coconut water (see Table 53).

*Dendrobium* Queen Sonia showed good growth in MS medium with 150 mg l\(^{-1}\) BP, same effect was seen even when 200 mg l\(^{-1}\) BP was used. But VW medium with 150 mg l\(^{-1}\) BP was better. The growth was moderate in KC medium. But in all media growth reached saturation at 150 mg l\(^{-1}\) BP, at lower levels(50and 100 mg l\(^{-1}\) BP) there was slow growth.

*Dendrobium* Emma White also responded similar to that of the other *Dendrobium* hybrid. In case of both the *Dendrobium* hybrids good rooting was noticed. In all the media rooting was good at 150 mg l\(^{-1}\) BP concentration. At 50 mg l\(^{-1}\) BP level small and thin roots developed which were fewer in number also. In MS medium with 200 mg l\(^{-1}\) BP green PLBs formed from the bases of the plants, which later on formed plants.

*Cattleya* Naomi Kerns showed good growth and rooting in VW medium with 150 mg l\(^{-1}\) BP but in MS medium with 150 mg l\(^{-1}\) BP the
growth and rooting was excellent. When the concentration of BP was increased to 200 mg l\(^{-1}\) there was no further improvement in the growth and rooting of plantlets. This effect was seen in both VW medium and MS medium. But in KC medium there was moderate growth observed.

In MS medium with 200 mg l\(^{-1}\) BP green PLBs were produced from the basal regions of the plantlets. There PLBs eventually developed into plantlets.

*Phalaenopsis* Queen Emma showed excellent growth and good rooting only in MS medium with 200 mg l\(^{-1}\) BP. The growth and rooting was poor in VW medium and KC medium. In MS medium with 50 mg l\(^{-1}\) BP both the growth and the rooting were slow with 100 mg l\(^{-1}\) BP and 150 mg l\(^{-1}\) it showed moderate and good growth respectively.

Effects of cytokinins, kinetin and 2,6-P in MS, VW and KC media supplemented with 150 ml l\(^{-1}\) BP, 500 mg l\(^{-1}\) activated charcoal 8.5 g l\(^{-1}\) agar, and 20 g l\(^{-1}\) sucrose on the growth of plantlets were studied (see Tables 52-55). Increase in the length of the plant, number of leaves and roots produced were observed at different levels of cytokinins. Plantlets measuring 1 cm long with 2 leaves and a few leaf primordia were inoculated on selected media:

*Dendrobium* Queen Sonia plantlets were cultured on VW medium. In the absence of kinetin the length of the plant was found to be 2.1 cm with 4.1 leaves and 5.1 roots were recorded. At 0.5 mg l\(^{-1}\) kinetin level the
length was found to be 2.2 cm with 4 leaves and 6 roots were recorded with the increase in kinetin level the growth, number of leaves of number of roots increased but saturation point was reached at 2.5 mg l\(^{-1}\) concentration, where 2.9 cm length and 4.6 leaves and 8.2 roots were observed. At 3 mg l\(^{-1}\) kinetin level the length was found to be 2 cm 3 leaves were seen but 8.2 roots were observed. 2iP was found to be better than kinetin.

At 0.5 mg l\(^{-1}\) 2iP level length of plants was 2.5 cm with 4.2 leaves and 5.2 roots were recorded. As the concentration of 2iP was increased the growth of the plantlets was improved. At 3 mg l\(^{-1}\) 2iP level length of the plants was found to be 3.2 with and 5.0 leaves. But leaves and roots were smaller as compared to those produced in the presence of kinetin and 9.0 roots were produced.

*Dendrobium* Emma White was cultured on VW medium. In the absence of kinetin the length of the plant was 2 cm and 4 leaves and 5 roots were recorded. At 2 mg l\(^{-1}\) kinetin level plant length was 2.8 cm and 4.5 leaves and 7.1 roots were recorded. At 2.5 mg l\(^{-1}\) 2iP length of the plant was 2.9 cm and 4.6 leaves and 8.2 roots were recorded. At 3.0 mg l\(^{-1}\) 2iP there was decrease in the growth, 1.8 cm length, 3 leaves and 9.0 roots were recorded. Roots produced were smaller and thin. Kinetin gave slightly better results than 2iP. At 0.5 mg l\(^{-1}\) 2iP 2.4 cm length and 4.2 leaves and 6.1 roots were recorded. At 2 mg l\(^{-1}\) 2iP level 2.8 cm length, and 4.2 leaves and 8.2 roots were produced. At 2.5 mg l\(^{-1}\) 2iP level, 3.0 cm
length 4.1 leaves and 10 roots were produced. At 3 mg l⁻¹ 2iP 3.1 cm length and 5 leaves and 10.6 roots were produced.

*Cattleya Naomi Kerns* plantlets were cultured on MS medium. In the absence of kinetin 3 cm length and 5.1 leaves and 3.1 roots were observed. At 1 mg l⁻¹ kinetin level 3.2 cm length and 5.3 leaves and 3.4 roots were recorded. At 2.0 mg l⁻¹ kinetin, 3.5 cm length and 5.5 leaves and 5.0 roots were recorded with the further increase in the kinetin level there was decrease in the length and number of leaves, but there was increase in the number of roots produced. At 3.0 mg l⁻¹ kinetin level 3.3 cm length and 5.1 leaves and 5.6 roots were observed. 2iP gave better results than kinetin. Length of the plants, number of leaves and number of roots increased with the increase in the concentration of 2iP. At 0.5 mg l⁻¹ 2iP level 2.9 cm length, and 5 leaves and 3.2 roots were observed. At 1.0 mg l⁻¹ 2iP level 3 cm length of the plantlet, 5.2 leaves and 3.2 roots were recorded at 2 mg l⁻¹ 2iP level 3.8 cm length, 5.4 leaves and 4.0 roots were recorded at 3.0 mg l⁻¹ 2iP level 3.9 cm length and 6.0 leaves and 5.3 roots were observed.

*Phalaenopsis Queen Emma* was cultured as MS medium. In the absence of kinetin 4.5 cm stem length and 2.0 leaves, and 2.2 roots were observed. As there was increase in the concentration of kinetin the growth of the plants was also increased. At 1.0 mg l⁻¹ kinetin level 1.6 cm length and 2.1 leaves and 2.6 roots were recorded. At 2 mg l⁻¹ kinetin level 1.9
cm length and 2.2 leaves and 3.6 roots were produced. At 3.0 mg l⁻¹ kinetin level 3.0 cm length, 4.0 leaves and 3.8 roots were recorded. 2iP gave better results than kinetin. At 1 mg l⁻¹ 2iP level 2.1 cm length and 2.2 leaves and 2.2 roots were recorded. At 2 mg l⁻¹ 2iP concentration 2.8 cm length and 3.2 leaves, 4.5 roots were observed. At 3 mg l⁻¹ 2iP level 3.4 cm length and 6.2 leaves and 5.4 roots were recorded.

Effects of different auxins, NAA, IBA and 2, 4-D on the growth of plantlets in different basal media with 1 mg l⁻¹ BAP, 150 ml l⁻¹ BP, 250 mg l⁻¹ activated charcoal 8.5g l⁻¹ agar and 20 gm l⁻¹ sucrose were studied. Length of the plantlets and number of leaves and roots produced were taken as parameters for this study (see Tables 56-59).

*Dendrobium* Queen Sonia was cultured on VW medium. At 2 mg l⁻¹ NAA level 2.5 cm length, 6.1 leaves and 4.2 roots were observed at 6 mg l⁻¹ NAA level 4.0 cm length, 8.0 leaves, and 6.1 roots were observed.

At 8 mg l⁻¹ NAA level 5.5 cm length 12.1 leaves, and 8.3 roots were recorded. In this case the roots produced were unusual and were thin and small.

**MULTIPUICATION OF PLANTLETS**

The rate of multiplication of plantlets used in different selected media was studied. There was a range of responses from normal growth of the plantlets to different degree of multiplication in terms of number of
plantlets produced at the end of 12 weeks of culture as compared to the number of plantlets originally used in the experiments (see Table 60 - 63).

Effects of auxins and cytokinins on the rate of multiplication of plantlets were studied by culturing the different plants on different selected media with 8.5 agar, 150 mg l\(^{-1}\) of coconut water, 20 mg l\(^{-1}\) sucrose and varied concentrations of NAA and BAP. Observation were made after 14 weeks of culture and multiplication ratio was calculated. Multiplication ratio is ratio of the total number of plants produced and the number of plants inoculated (see Tables 63-66, figs. 8, 9, 15, 21, 34, 37, 38, 42, 44, 61, 62, 62, 100, 103 and 105).

It was found that both NAA and BAP were necessary for multiplication. Rate of multiplication increased with the increase in the BAP concentration up to certain levels in all cases. But the multiplication ratio decreased as there was further increase in the concentration of the growth regulators. This clearly indicates that each hybrid requires a specific combination of growth regulators for proper growth. In the absence of BAP and the presence of only NAA there was little multiplication. Multiplication always occurred by the formation of side shoots from the base of the plants in the medium.

*Dendrobium* Queen Sonia was cultured on VW medium. In the presence of 10 mg l\(^{-1}\) NAA and no BAP 1.8 multiplication ration (MR) was recorded. In the presence of 1 mg l\(^{-1}\) BAP and 6 mg l\(^{-1}\) NAA 2.5 MR was
observed. At 2 mg l\(^{-1}\) BAP and 4 mg l\(^{-1}\) NAA level. MR was found to be 7. At 4 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) Maximum MR (12.5) was recorded and this was found to be the best ratio of concentrations of BAP and NAA for good multiplication with proper growth. At 6 mg l\(^{-1}\) BAP and no NAA 6.5 MR was recorded and the MR was 6 at 8 mg l\(^{-1}\) BAP and 0 mg l\(^{-1}\) NAA. But when the concentration of both NAA and BAP exceeded 8 mg l\(^{-1}\) plants were found to be abnormal, showing small and narrow leaves and thin roots.

*Dendrobium* Emma White was cultured on VW medium. In the absence of BAP with the increase in the NAA concentration from 1 to 10 mg l\(^{-1}\) there was slow increase in the MR from 1 to 1.9. MR was 1.2 at 2 mg l\(^{-1}\) NAA and no BAP. But with 1 mg l\(^{-1}\) BAP and 2 mg l\(^{-1}\) NAA MR was 2.5 and it was 3 at the same level of BAP (1 mg l\(^{-1}\)) and 4 mg l\(^{-1}\) NAA. At 2 mg l\(^{-1}\) BAP and 4 mg l\(^{-1}\) NAA concentration, MR was 6. Maximum MR for this hybrid was 11.5, recorded at 4 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) concentration. MR was 9 at 4 mg l\(^{-1}\) BAP and 2 mg l\(^{-1}\) NAA concentration. MR was 7 in the absence of NAA and in the presence of 6 mg l\(^{-1}\) BAP. At 8 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) NAA MR was 5. MR was 1.5 at 10 mg l\(^{-1}\) BAP and 10 mg l\(^{-1}\) NAA. Results indicate that both NAA and BAP are necessary for multiplication.

*Cattleya* Naomi Kerns was cultured on MS medium. In the absence of BAP and with only NAA, the MR was very low. MR increased
from 1.4 to 1.9 with the increase in the concentration of NAA from 0 to 10 mg l\(^{-1}\). MR was 2.5 at 1 mg l\(^{-1}\) each of NAA and BAP. Maximum MR (9.5) was observed at a combination of 2 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) NAA. It was 9 at 4 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) NAA concentration. At 8 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) NAA concentration 3.5 MR was observed. At 10 mg l\(^{-1}\) BAP and 2 mg l\(^{-1}\) NAA 2.5 MR was recorded and it was 1.8 at 10 mg l\(^{-1}\) each of NAA and BAP. There was increase in the MR with increase in BAP upto 4 mg l\(^{-1}\), but with the further increase in BAP concentration, MR decreased. A specific combination of concentrations of NAA and BAP was necessary for proper multiplication of plants.

Phalaenopsis Queen Emma was cultured on MS medium. In the absence of BAP and with only NAA ranging between 1 mg l\(^{-1}\) to 6 mg l\(^{-1}\) there was no multiplication. MR was 1.2 and 1.9 at 8 mg l\(^{-1}\) and 10 mg l\(^{-1}\) NAA concentrations respectively. MR was ranging between 1 to 1.9 when 1 mg l\(^{-1}\) BAP and 1 - 10 mg l\(^{-1}\) NAA were used in the medium. At 2 mg l\(^{-1}\) BAP and 4 mg l\(^{-1}\) NAA the MR was 2 and it was 2.5 when 4 mg l\(^{-1}\) BAP 2 mg l\(^{-1}\) NAA were used in the medium. 4.5 MR was observed at 6 mg l\(^{-1}\) BAP and 4 mg l\(^{-1}\) NAA concentration. MR for this hybrid was found to be a maximum of 9.5 at 8 mg l\(^{-1}\) BAP and 4 mg l\(^{-1}\) NAA concentration. At 10 mg l\(^{-1}\) BAP and 1 mg l\(^{-1}\) NAA MR was 9 and it was 1.2 at 10 mg l\(^{-1}\) each of NAA and BAP. Phalaenopsis hybrid showed the requirement higher concentrations of NAA and BAP for multiplication as compared to other
orchid hybrids studied. Though both these growth regulators were necessary the requirement of BAP was more for multiplication of plantlets.

At 10 mg l⁻¹ NAA level also plants showed unusual features. Stem length was 9.0 cm with 9.4 narrow leaves, with long internodes, the leaves were pale green in colour. 8.5 roots were produced but the roots were thin and small. Growth was moderate in the presence of IBA. At 2 mg l⁻¹ IBA 2.4 cm length and 4 leaves and 3.1 roots were observed.

At 6 mg l⁻¹ IBA level 3.3 cm length, 5.1 leaves and 4.0 roots were recorded. At 8 mg l⁻¹ IBA level 5.0 cm growth, 6.5 leaves and 5.0 roots were recorded. At 10 mg l⁻¹ IBA, 7.3 cm length, 6.8 leaves and 5.2 roots were observed. The growth increased with the increase in the IBA level even the number of leaves and roots also increased, but in the presence of 8 and 10 mg l⁻¹ IBA unusual plants were produced. Growth was moderate in the presence, of 2, 4D also. But 6, 8, and 10 mg l⁻¹ level induced the formation of unusual plants. At 2 mg l⁻¹ 2, 4D, 2.5 length, 4.1 leaves and 2.0 roots were observed.

At 6 mg l⁻¹ 2, 4-D level, 6.2 leaves, 3.0 roots were recorded. At 10 mg l⁻¹ 2, 4-D 7.3 cm length, 6.6 leaves and 1.1 roots were recorded. Though the stem length and number of leaves increased with the increase in 2, 4-D concentration, the number of roots decreased. Of all the auxins tried at different levels, Dendrobium Queen Sonia showed good, healthy and normal growth without any abnormalities at 6 mg l⁻¹ NAA level.
Dendrobium Emma White was cultured on VW medium. At 2 mg l\(^{-1}\) NAA level, 3 cm length, and 6.0 leaves and 4.0 roots were recorded. At 4.0 mg l\(^{-1}\) NAA level, 4.1 cm length, 8.2 leaves, 6.1 roots were observed. At 6 mg l\(^{-1}\) NAA healthy and normal plants were formed. At this level 4.5 cm length 9.1 leaves, and 8.2 roots were observed. At 8 mg l\(^{-1}\) NAA 6.0 cm length 12.3 leaves and 8.3 roots were recorded. But both leaves and roots were unusual. At 10 mg l\(^{-1}\) NAA level 8.5 cm length 10.1 leaves and 5.4 roots were observed. The growth was moderate in the presence of IBA. At 2.0 mg l\(^{-1}\) IBA level 2.5 cm length 4.1 leaves and 2.1 roots were observed. At 6 mg l\(^{-1}\) NAA level 3.5 cm length was recorded with 5.4 leaves and 4.2 roots. At 10 mg l\(^{-1}\) IBA, 6.5 cm length was recorded with 6.2 leaves and 4.4 roots, but both leaves and roots were found to be abnormal. 2, 4-D induced growth only at 2 and 4 mg l\(^{-1}\) level. At 6, 8 and 10 mg l\(^{-1}\) 2, 4-D unusual plants were formed. At 2 mg l\(^{-1}\) 2, 4-D, 2.5 cm length 4.2 leaves and 2.2 roots were noticed. At 4 mg l\(^{-1}\) 2, 4-D, 3.5 cm length was recorded with 6.3 leaves and 2.2 roots. At 10 mg l\(^{-1}\) 2, 4-D level, 7.5 cm length was recorded with 6.8 leaves and 1.0 root. Increase in 2, 4-D level increased the growth and number of leaves but the number of roots reduced 2, 4-D was not found to be a good growth regulator for the growth of plants. NAA was found to be the growth regulator best followed by IBA.
Cattleya Naomi Kerns plantlets were cultured on MS medium. NAA was found to be better auxin than IBA and 2, 4-D. IBA gave moderate results. Though 2, 4-D gave better results than IBA the plants obtained by using higher concentrations of 2, 4-D were unusual. At 2 mg l⁻¹ NAA level 3.1 cm length was observed and 4.0 leaves and 2.2 roots were produced. At 4 mg l⁻¹ NAA level length of the plant was 4 cm with 6.1 leaves and 3.2 roots. Growth was better with the increase in the NAA level. At 10 mg l⁻¹ NAA level the length of the plants was 7.0 cm with 6.2 leaves and 5.1 roots. But the plants were abnormal with long internodes. At 2 mg l⁻¹ IBA level the length of plant was 2.8 cm with 4.2 leaves and 2.0 roots. Growth improved with the increase in IBA level. At 8 mg l⁻¹ IBA concentration, the length of plant was 4.2 cm with 4.3 leaves and 4.2 roots at 10 mg l⁻¹ IBA the length was 5.1 cm and 4.4 leaves and 4.2 roots were produced. In this case, though there was increase in the stem length by 1 cm the increase in the number of leaves was very less, and it was only 0.1. This leads to increase in the internodal length. The other observation was that the plants produced under the influence of higher levels of IBA were with smaller with narrower leaves as compared to those produced in the presence of NAA. At 2 mg l⁻¹ 2, 4-D level, the length of plant was 2.2 cm with 3.1 leaves and 2.1 roots. At 6 mg l⁻¹ concentration, the length was 3.4 cm and 4.2 leaves and 4.0 roots were produced. At 10 mg l⁻¹ 2, 4-D level, the length was 5.6 cm and 5.7 leaves and 4.3 roots were produced.
Though the growth was better in terms length of the stem and number leaves and roots in the presence of 2, 4-D than in the presence of IBA, the sizes of leaves and roots were smaller with unusual features.

*Phalaenopsis* Queen Emma was cultured in MS medium. NAA was a better auxin. Plants showed excellent growth in the presence of 10 mg l\(^{-1}\) NAA. It showed higher requirement of NAA than the orchids studied. IBA induced moderate growth even at higher (10 mg l\(^{-1}\)) level 2, 4-D induced slow growth at all levels studied. At 2 mg l\(^{-1}\) NAA concentration, the plant length was 2.1 cm with 2.1 leaves and 2.0 roots. At 6 mg l\(^{-1}\) NAA concentration the length was 3.4 cm and 2.2 leaves and 5.2 roots were produced. At 10 mg l\(^{-1}\) 3.7 cm length was recorded with 4.1 leaves and 8.1 roots. At 2 mg l\(^{-1}\) IBA the plant length was 1.5 cm and 2 leaves and 2 roots were observed. Hence there was no increase in the number of leaves but 2 roots were formed and stem length increased. At 6 mg l\(^{-1}\) IBA concentration the length was 1.8 cm, and 2.2 leaves and 2.1 were observed. At 10 mg l\(^{-1}\) IBA 2.2 cm length was recorded and 4.2 leaves and 3.3 roots were observed. At 2 mg l\(^{-1}\) 2, 4-D concentration the plant length increased to 1.5 cm and 1.9 roots were formed but there was no increase in the number of leaves. At 6 mg l\(^{-1}\) 2, 4-D concentration the plant length was 2 cm. The number of leaves marginally increased to 2.1 and 2.1 roots were observed. At 10 mg l\(^{-1}\) 2, 4-D, the plant length was 1.5 cm with 2.2 leaves and 1.9 roots. There was slight increase in the plant
length with the increase in 2, 4-D concentration but above 8 mg l\(^{-1}\) level there was further decline in the growth of the plants in terms of length. Increase in the number of leaves is very little and the root formation was also poor in the presence of 2,4-D.

**GROWTH AND MAINTENANCE OF REGENERATED PLANTS**

Bottles containing plants to be hardened were kept in the green house for 3-4 days. Plants which have grown sufficiently in the media were taken out and grown in the green house. In case of *Dendrobium* and *Cattleya* 3-4 cm high plants were used for hardening. Whereas in case of *Phalaenopsis* 2-3 cm high plants were hardened.

All orchids were initially grown in community pots for 3-4 weeks, during that time plants acclimatize to green house conditions. Different media were used for community potting. Potting medium with charcoal and very small clay pieces was better. Coconut husk pieces and moss were used together in equal proportion, it induced good rooting and growth of the plants but mortality rate was high as moss retained too much water and caused decay of plants (see figs. 22-24, 48, 49, 73, 74, 107, 108 and 109).

Plants were taken out from the community pots after 3-4 weeks and grown in individual pots, with only one plant in each pot. The following types of potting media were used: (a) Charcoal pieces (b) Broken burnt clay pieces (c) Brick pieces (d) Charcoal and clay pieces (e) Charcoal,
clay pieces and moss (f) Charcoal, clay pieces and coconut husk pieces (g) Coconut husk pieces (h) Rock wool and clay pieces (i) Clay balls and coconut husk pieces (j) Fern blocks (k) Wooden blocks and moss and (l) Split coconut pericarp and coir.

Charcoal pieces of about 2 cm in diameter were best for rooting and growth. Brick pieces used with charcoal, helped in retaining moisture. 5:1 ratio of charcoal and brick pieces was ideal for Cattleya hybrid. Dendrobium hybrid showed good growth in the medium containing clay balls and coconut husk pieces. Coconut husk was used after removing tannin contents by soaking in water for 10 - 15 days. Phalaenopsis hybrid showed good growth in the potting medium containing tree fern blocks.

All potted plants were kept in green house, maintaining high humidity. They were provided with nutrients in the form of foliar spray. A mixture of NPK (Nitrogen 30 gm l$^{-1}$ phosphorous 20 g l$^{-1}$ potassium 20 g l$^{-1}$) and micro nutrients of MS medium were dissolved in one liter of water. 0.1% of this mixture was prepared by diluting and sprayed on the plants once in a week. Watering was done once in a week.