CHAPTER THREE

OBSERVATIONS
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3.1 HORMONE INDUCED BUD FORMATION IN SUCKERS.

The application of 40 mg l$^{-1}$ BAP to the apex of the decapitated nendran and poovan suckers induced 4-5 shoots from each sucker within a period of 3 months. The shoots so obtained, later on produced roots from base, forming full-fledged shoots. Such shoots on isolation and transfer to the field showed that only 38% of the nendran plants and 37.5% of the poovan plants produced by this method alone got established in the field. Of those established on the field 42% of the nendran plants and 33.33% of the poovan plants alone grew to a height of 1-2 meters, but did not produce flowers and fruits, while others did not grow even to one meter.

3.2 IN VITRO CULTURE STUDIES

*In vitro* culture studies on the Nendran and Poovan cultivar varieties of banana plant using shoot tip and inflorescence apex explants showed the following results.

3.2.1 INFLORESCENCE TIP CULTURE OF NENDRAN VARIETY

*In vitro* cultures could be readily established from nendran inflorescence tips. The explants cultured on MS medium with 30-40 mg l$^{-1}$ sucrose and 1 to 5 mg l$^{-1}$ BAP initiated growth response and induced growth of the explants within 30 days of culture.

The medium containing 1 mg l$^{-1}$ BAP initiated only growth of the explants. The culture medium with 2 mg l$^{-1}$, 3 mg l$^{-1}$ and 5 mg l$^{-1}$ BAP induced an initial response of increase in length and breadth of the explants within 30 days of
incubation in the culture medium. Further growth response was obtained only in medium with 3 mg/l and 5 mg/l BAP. The response was slow in the growth media containing 5 mg/l BAP and required a longer period of 90 days to initiate growth of few shoots. Multiple shoots were produced in medium with 3 mg/l and 5 mg/l BAP. Only 35 percent of the explants responded in the medium with 5 mg/l BAP inducing an average number of 15 buds in 120 days of culture. But the leaves of the plants produced were thick and fleshy showing vitrification to a certain extent. Further the shoots produced were dwarf in nature. The explants in the medium with 3 mg/l BAP demonstrated an average number of 8 buds with a response of 78% (Fig.1, Table 3.1). The shoots produced were very weak in nature and took a further period of 150 days for elongation.

**TABLE 3.1 - RESPONSE OF INFLORESCENCE TIP EXPLANTS OF NENDRAN VARIETY CULTURED ON MS MEDIUM WITH 1-5 mg/l BAP.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/l BAP</td>
</tr>
<tr>
<td></td>
<td>Days of observation</td>
</tr>
<tr>
<td></td>
<td>30 60 90 120</td>
</tr>
<tr>
<td>Average no. shoots</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>% of response</td>
<td>100 80 72 68</td>
</tr>
</tbody>
</table>

Later the MS medium was tried with a modified combination of vitamins by adding 0.003 mg/l folic acid, 0.005 mg/l biotin and riboflavin each, 0.001 mg/l each of thiamine HCl, pyridoxine HCl and nicotinic acid (this medium will be referred hereafter as MS1). The explants cultured on 2 and 3 mg/l BAP initiated growth
response after 30 days of culture. On further incubation in the medium with 2 mg/l BAP induced only an average of one shoot each. On the contrary, the response pattern in medium with 3 mg/l BAP was quite different. The inflorescence tip explant turned green and initiated growth within 30 days of culture in the MS1 medium with 3 mg/l BAP (Fig. 2). On further incubation 4 buds developed from the axil of the bracts (Fig. 3). The bud number increased to 8 (Fig. 4) within 90 days of culture. Further incubation in the same medium for a total period of 120 days showed induction of 15 buds per explant (Table 3.2 and Fig. 5). The average percentage of responding explants that gave good response at 120 days of culture in this medium was 83.

### TABLE 3.2 - MS NUTRIENTS WITH NEW COMBINATIONS OF VITAMINS (MS1) SUPPLEMENTED WITH 30g/l SUCROSE AND 2-3mg/l BAP.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/l BAP</td>
</tr>
<tr>
<td></td>
<td>Days of observation</td>
</tr>
<tr>
<td></td>
<td>30 60 90 120</td>
</tr>
<tr>
<td>Average no. of shoots</td>
<td>0 1 1 1</td>
</tr>
<tr>
<td>% of response</td>
<td>100 90 86 70</td>
</tr>
</tbody>
</table>

Since the medium with 3 mg/l BAP demonstrated rather good response further experiments were designed with this media supplemented with 30-50 g/l sucrose. The medium containing 40 g/l sucrose and 2 mg/l BAP demonstrated only an average number of one shoot each. Conversely the inflorescence tip explants incubated on MS1 medium containing 40 g/l sucrose and 3 mg/l BAP initiated the
growth of the explant within 30 days of culture (Fig. 6). Further incubation in the
same medium induced an average number of eight buds (Fig. 7) within 60 days and
14 buds after 90 days (Fig. 8) and 18 buds after 120 days of culture (Fig. 9
& Table 3.3).

TABLE 3.3 - RESPONSE OF EXPLANTS ON MS1 MEDIUM
CONTAINING \(3\text{mg}l^{-1}\) BAP AND DIFFERENT
CONCENTRATIONS OF SUCROSE.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of sucrose</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 gl(^{-1})</td>
<td>40 gl(^{-1})</td>
<td>50 gl(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Days of observation</td>
<td>30 60 90 120</td>
<td>30 60 90 120</td>
<td>30 60 90 120</td>
<td></td>
</tr>
<tr>
<td>Average no. of shoot</td>
<td>1 4 8 15</td>
<td>4 8 14 18</td>
<td>1 2 5 6</td>
<td></td>
</tr>
<tr>
<td>% of response</td>
<td>100 90 85 83</td>
<td>100 94 90 88</td>
<td>92 78 47 35</td>
<td></td>
</tr>
</tbody>
</table>

The shoots so produced after 150 days were excised into groups of 4-6
shoots and sub cultured to MS1 medium with \(3\text{mg}l^{-1}\) IBA and \(1.5\text{mg}l^{-1}\) BAP for
further multiplication and elongation of shoots. The cluster of shoots elongated
within 45 days of sub culture, along with further multiplication of shoots (Fig. 10).
A few shoots were rooted in the elongation medium. Individual elongated shoots
excised after 60 days of subculture and transferred to the MS1 medium with \(3\text{mg}l^{-1}\)
IBA and \(0.5\text{mg}l^{-1}\) BAP (Fig. 11) rooted within 45 days of sub culture. Each of the
shoots developed an average number of 3-4 adventitious roots with root hairs in 45
days of transfer (Fig. 12). 90 percent of the rooted shoots transferred to the field
after hardening and acclimatization employing the standard method of hardening
(Fig. 13) were established in the field as healthy plants (Fig. 14).
On the other hand in the case of explants cultured on MS1 medium containing 40gl⁻¹ sucrose and 5 mgl⁻¹ BAP an average number of 38 shoots were produced per explant in 120 days of culture. But the percentage of response was only 35 (Fig.15). On transfer to medium containing 3mg⁻¹ IBA and 1mg⁻¹ BAP only 30% of shoots showed elongation within 40 days of transfer and no shoots produced roots. Addition of 0.5mg⁻¹ gibberellin to the medium increased the percentage of elongated shoots to 56%. The elongated shoots on transfer to medium containing 3mg⁻¹ IBA and 0.5mg⁻¹ BAP induced roots within 35 days of sub culture (Fig.16). The number of roots was increased to 2-3 within 45 days of culture. Addition of 370 mg⁻¹ NaH₂PO₄ to the MS1 medium with 3mg⁻¹ BAP did not show any enhancement in the result.

3.2.2 INFLORESCENCE TIP CULTURE OF POOVAN VARIETY

Excised inflorescence tips of cv. Poovan cultured on MS media containing 2-5 mg⁻¹ BAP and 30gl⁻¹ sucrose showed an initial growth of the apical bud in all the media combinations tried by inducing the growth of the bracts. These bracts turned green on transfer to photoperiodic conditions. Small flower buds appeared in clusters in the axil of the bracts in medium containing 2mg⁻¹ and 3mg⁻¹ BAP but no shoot formation was observed (Fig.17). In MS1 medium with 2.5mg⁻¹ BAP also the explants turned green within 40 days of culture. MS1 medium containing 2mg⁻¹ BAP induced small clusters of flowers in the axil of the lowermost bracts within a period of 90 days of culture. On the contrary, the medium with 3mg⁻¹ BAP induced initial growth of small flowers from the axil of the bracts along with shoot formation in the axil of the lower most bracts as observed after 120 days of
culture (Fig. 18). The medium with $5 \text{mg}^{-1} \text{BAP}$ showed almost the same response, but the percentage of responding explants was much less.

Experiments with addition of adenine sulphate to MS1 medium altered the pattern of response. In the medium containing 2, 3, and $5 \text{mg}^{-1} \text{BAP}$ and $30 \text{mg}^{-1}$ sucrose with 100, 160, 200 $\text{mg}^{-1}$ adenine sulphate was tried and the explants responded differently in the different combinations. The MS1 medium containing $2 \text{mg}^{-1}$, $3 \text{mg}^{-1}$ BAP along with $100 \text{mg}^{-1}$, $200 \text{mg}^{-1}$ adenine sulphate showed an initial response within a period of 40 days, but the number of shoots produced were very few (Fig. 19). Even though the shoots produced were few they got developed and found elongating within 120 days of culture. (Fig. 20) But the medium with $3 \text{mg}^{-1}$ BAP supplemented with $150 \text{mg}^{-1}$ adenine sulphate showed active initiation of growth of the explant (Fig. 21). Later a special pattern of response is observed in the inflorescence tips of poovan cultured on MS1 medium with $3 \text{mg}^{-1}$ BAP and $160 \text{mg}^{-1}$ adenine sulphate. The explants incubated on this culture medium initially showed flourishing growth of the bract so that the responding explant gives the appearance of an opening flower bud after 30 days of culture (Fig. 21). A few whitish globular protocorm like bodies emerge out from the axil of the lower bracts which grew further (Fig. 22). On subculture to the same medium, after 60 days of culture, meristems differentiate from these whitish bodies, each one of which differentiated into a shoot (Fig. 23). The cultured explants developed an average number of 18 buds in a period of 120 days of culture (Fig. 24), which further increased to 32 in 150 days of culture (Table- 3.4, Fig. 25).
TABLE 3.4 - RESPONSE OF POOVAN INFLORESCENCE TIP EXPLANTS IN MS1 MEDIUM CONTAINING 3mgl⁻¹ BAP AND DIFFERENT CONCENTRATIONS OF ADENINE SULPHATE

<table>
<thead>
<tr>
<th>Adenine sulphate in mgL⁻¹</th>
<th>Average number of shoots produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 days</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>160</td>
<td>12</td>
</tr>
<tr>
<td>200</td>
<td>2</td>
</tr>
</tbody>
</table>

The shoot buds produced in culture did not elongate in the same medium. Hence, they were cut into segments of 4-6 shoots and transferred to MS1 medium containing 3mgl⁻¹IBA and 1.5mgl⁻¹ BAP for further multiplication and shoot elongation. The shoots multiplied and many of them elongated within 60 days of transfer (Fig. 26). A few shoots formed roots within the elongation medium. Individual shoots excised and transferred to the rooting medium containing 3mgl⁻¹IBA and 0.5mgl⁻¹ BAP developed 3-4 roots within 45 days of subculture (Fig. 27). Addition of 370mgl⁻¹ NaH₂PO₄ to the MS1 medium containing 3mgl⁻¹ BAP did not induce any pronounced change in the response. 80% of the hardened plants transferred to the field established in the field showed normal growth pattern and morphology (Fig. 28). Explants cultured on MS1 medium with 3mgl⁻¹ BAP, 160mgl⁻¹ adenine sulphate and 370mgl⁻¹sodium dihydrogen orthophosphate showed slow response and produced 4-5 buds concomitant with gradual withering of the explant tip (Fig. 29).

Various attempts with explants of different sizes have shown that explants having an average size of 20.5x1.75 mm produced an average number of 32 buds
in 150 days of culture, while the explant of size 12x13 mm size needed 180 days of incubation to induce the same number of buds.

3.2.3 SHOOT TIP CULTURE OF NENDRAN.

Shoot tip explants of nendran showed different responses in all the media tried. Shoot tip explants containing the meristematic dome consisting of a few cell layers surrounded by 6-8 leaf primordia were incubated on MS medium with 2-5 mg/l BAP and 30 g/l sucrose. The explants on MS medium with 30 g/l sucrose and 2.5 mg/l BAP, showed only initiation of growth. But explants in 3 mg/l BAP in MS medium showed growth of the apical bud followed by initiation of leaf opposed buds in 120 days of culture (Table 3.5).

**TABLE 3.5 - RESPONSE OF NENDRAN SHOOT TIP EXPLANTS ON MS MEDIUM WITH 2.3 AND 5 mg/l BAP**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/l BAP</td>
</tr>
<tr>
<td>Days of observation</td>
<td>Days of observation</td>
</tr>
<tr>
<td>30 60 90 120</td>
<td>30 60 90 120</td>
</tr>
<tr>
<td>Average no. of shoots</td>
<td>1 1 2 2</td>
</tr>
<tr>
<td>% of response</td>
<td>70 50 40 20</td>
</tr>
</tbody>
</table>

Since the trial with MS nutrients did not give good response, experiments were designed with modified MS medium (MS1 medium) containing 2, 3, 5 and 10 mg/l BAP. MS1 medium with 2 mg/l BAP showed an initial response of growth of the apical bud followed by induction of 2 buds in 120 days of culture.
explants in $\text{5mg}\text{l}^{-1}$ BAP induced certain outgrowths from the lower part of the explant which grew from the base of the explant and developed into corm like structures. Such responding explants after 60 days of culture in the initiation medium on subculture to the medium containing $\text{0.5mg}\text{l}^{-1}$ IBA and $\text{3mg}\text{l}^{-1}$ BAP developed shoots within 60 days of culture (Fig.30). On the other hand even if these corm like structures were excised, isolated and cultured on MS1 medium with $\text{0.5mg}\text{l}^{-1}$ IBA and $\text{3mg}\text{l}^{-1}$ BAP, shoots were regenerated from it within 120 days of subculture (Fig.31). But the shoots produced were dwarf in stature, with thick fleshy leaves (Fig.32) and difficult to elongate. The percentage frequency of responding explants was only 35 (Table 3.6) at 120 days of culture in $\text{5mg}\text{l}^{-1}$ medium. Conversely, the explants cultured on the $\text{3mg}\text{l}^{-1}$ BAP containing medium induced 8 buds in 62% of the cultured explants and the shoots produced were normal and healthy in appearance. The explants incubated on medium containing $\text{10mg}\text{l}^{-1}$ gradually turned brown, darkend and become dead.

**TABLE 3.6 - RESPONSE OF NENDRAN SHOOT TIP EXPLANTS ON MS1 MEDIUM WITH 2, 3 AND 5 mg$l$$^{-1}$ BAP.**

<table>
<thead>
<tr>
<th>Concentration of BAP</th>
<th>Parameters</th>
<th>2 $\text{mg}\text{l}^{-1}$</th>
<th>3 $\text{mg}\text{l}^{-1}$</th>
<th>5 $\text{mg}\text{l}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of observation</td>
<td>Days of observation</td>
<td>Days of observation</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Average no of shoots</td>
<td>30 60 90 120</td>
<td>30 60 90 120</td>
<td>30 60 90 120</td>
<td></td>
</tr>
<tr>
<td>% of response</td>
<td>98 89 64 42</td>
<td>100 92 85 62</td>
<td>84 78 62 35</td>
<td></td>
</tr>
</tbody>
</table>

Since, the MS medium containing $\text{3mg}\text{l}^{-1}$ BAP was found showing better response, further experiments were designed with varying concentrations of
NaH₂PO₄. The MS1 medium with 3mg/l BAP and 30g/l sucrose with an addition of 400mg/l of sodium dihydrogen orthophosphate showed an appreciable increase in response during the early days of culture by inducing 4-6 sprouting buds, within a period of 60 days of culture but later by the exudation of phenolic compounds turned dark, became blackened and dead within 90 days of culture.

But when the amount of sodium dihydrogen orthophosphate was reduced to 370mg/l in MS1 medium supplemented with 3mg/l BAP, only 8 buds were induced from the axil of the lower tracts within 60 days of culture. The responding explants on subculture to same medium, after 90 days showed an average number of 12 buds (Fig.33). The average number of buds induced was enhanced to 14 with an average percentage response of 62 in 120 days of culture (Table 3.7). Further reduction in the concentration of NaH₂PO₄ only reduced the number of buds produced.

**TABLE 3.7 - RESPONSE OF NENDRAN SHOOT TIP EXPLANTS ON MS1 MEDIUM SUPPLEMENTED WITH 2, 3 AND 5 mg/l BAP AND 370 mg/l NaH₂PO₄**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/l</td>
</tr>
<tr>
<td></td>
<td>Days of observation</td>
</tr>
<tr>
<td></td>
<td>30       60         90   120</td>
</tr>
<tr>
<td>Average no of shoots</td>
<td>0   1           1      2</td>
</tr>
<tr>
<td>% of response</td>
<td>94  80          68     54</td>
</tr>
</tbody>
</table>

Addition of 160 mg/l adenine sulphate to the MS1 medium containing 370 mg/l sodium dihydrogen orthophosphate and 3mg/l BAP did not show any
positive response, rather the explants, even though initiated growth later on turned black and became dead.

With regard to the explants cultured on MS1 containing 370 mg l\(^{-1}\) BAP NaH\(_2\)PO\(_4\) and 5 mg l\(^{-1}\) BAP the response started by initiating development of the apical bud, followed by development of 3-4 corm like outgrowths from the leaf opposed position of the explant at the basal region. These structures grew and developed into corm like structures. These structures on excision and transfer to media with 0.5 mg l\(^{-1}\) IBA initiated development of 6-8 shoots within 60 days of subculture. The shoots produced in media with 5 mg l\(^{-1}\) BAP on excision and transfer to media with 3 mg l\(^{-1}\) IBA, 1.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) gibberellin, showed elongation in 60% of the shoots after 85 days of subculture.

The buds obtained in medium with 3 mg l\(^{-1}\) BAP excised into groups of 4 to 6 each and sub cultured on medium with 3 mg l\(^{-1}\) IBA and 1.5 mg l\(^{-1}\) BAP got elongated and multiplied. A few such elongated shoots produced one or two roots within 40 days of incubation. Those elongated shoots which did not have roots were excised and transferred to the rooting medium containing 3 mg l\(^{-1}\) IBA and 0.5 mg l\(^{-1}\) BAP. These shoots produced 3-4 roots with well-developed root hairs within 60 days of culture. The plants so obtained, hardened and transferred to the field showed 70% establishment in the field with normal growth pattern and morphology. Similarly the shoots elongated in medium with 3 mg l\(^{-1}\) IBA 1.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) GA\(_3\) were also rooted in medium with 3 mg l\(^{-1}\) IBA and 0.5 mg l\(^{-1}\) BAP, hardened and transferred to the field. Only 42% of such plants showed establishment in the field.
3.2.4 SHOOT TIP CULTURE OF POOVAN

Experiments were conducted with MS and MS1 media. Shoot tip explants cultured on MS medium with 2 and 3 mg/l BAP induced growth of the explant resulting in single shoot formation with an average of 78% and 82% response respectively (Fig.34). Conversely, the shoot tip explant cultured on medium with 5 mg/l BAP produced single shoot only on 38% of the explants, while others turned dark and became dead subsequently.

Since the MS medium was not found to be suitable for inducing a good response, the MS1 medium was tried with various combinations of BAP. In the experiments with 2, 3 and 5 mg/l BAP, the explants cultured on medium supplemented with 3 mg/l BAP responded positively initiating growth of the explants within 30 days of inoculation. On further incubation in the same medium 68% of the explants incubated in medium with 3 mg/l BAP produced an average number of 12 shoots per explant within 120 days of culture (Fig.35). Further incubation extending to 150 days enhanced the number of buds to 14. On the other hand only 25% of the explants produced multiple shoots giving an average number of 6-8 shoots in medium with 5 mg/l BAP (Table 3.8 and Fig.36) in 120 days of culture. The explants in medium containing 2 mg/l BAP induced only 3 shoots in 150 days of culture.
### TABLE 3.8 - RESPONSE OF POOVAN SHOOT TIP EXPLANTS ON MS1 MEDIUM WITH 3 AND 5mg/l BAP.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/l</td>
</tr>
<tr>
<td></td>
<td>Days of observation</td>
</tr>
<tr>
<td></td>
<td>30 60 90 120</td>
</tr>
<tr>
<td>Average no. of shoots</td>
<td>5 6 10 12</td>
</tr>
<tr>
<td>% of response</td>
<td>98 80 76 68</td>
</tr>
</tbody>
</table>

Shoots thus produced on transfer to medium containing 3 mg/l IBA and 0.5mg/l BAP induced 2-3 roots within 50 days of subculture. Addition of 370mg/l sodium dihydrogen orthophosphate to medium containing 3mg/l BAP enhanced the strength and health of the buds besides increasing the number of buds (Table 3.9).

### TABLE 3.9 - RESPONSE OF POOVAN SHOOT TIPS ON MS1 MEDIUM WITH 3mg/l BAP AND DIFFERENT CONCENTRATIONS OF NaH₂PO₄

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of NaH₂PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300 mg/l</td>
</tr>
<tr>
<td></td>
<td>Days of observation</td>
</tr>
<tr>
<td></td>
<td>30 60 90 120</td>
</tr>
<tr>
<td>Average no. of shoots</td>
<td>2 4 8 9</td>
</tr>
<tr>
<td>% of response</td>
<td>94 82 70 54</td>
</tr>
</tbody>
</table>

Further modification of medium by supplementing it with 160mg/l adenine sulphate demonstrated an increase in the length and diameter of the cultured...
explant within 40 days of incubation (Fig.37). The shoot tip explants of poovan showed a special pattern of bud induction in this medium. Certain whitish globular outgrowths developed from the leaf opposed region of the shoot tips (Fig.38), which grew and developed into protocorm like bodies. Two to three protocorm like bodies develop from a single shoot tip explant (Fig.39). Shoot meristems developed from the peripheral cell layers of these bodies which later on differentiate into shoots (Fig.40). Since 2-3 protocorm like bodies are developed from a single explant, 2-3 cluster of shoots can be found in a responding explant at a later stage (Fig.41). The average number of shoots produced per explant within 120 days of culture was 14 (Fig.42). The shoot number increased to 18 within a period of 160 days of incubation in culture conditions (Table 3.10).

**TABLE 3.10 - RESPONSE OF POOVAN SHOOT TIP EXPLANTS ON MS1 WITH 3mg l⁻¹ BAP, 370 mg l⁻¹ Na₂HPO₄ AND DIFFERENT CONCENTRATIONS OF ADENINE SULPHATE**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of adenine sulphate</th>
<th>120 mg l⁻¹</th>
<th>160 mg l⁻¹</th>
<th>200 mg l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of observation</td>
<td>120</td>
<td>160</td>
<td>120</td>
</tr>
<tr>
<td>Average no. shoots</td>
<td></td>
<td>4</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>% of response</td>
<td></td>
<td>70</td>
<td>48</td>
<td>72</td>
</tr>
</tbody>
</table>

The shoots so obtained cut into segments of 4-6 shoots and subcultured to medium supplemented with 3mg l⁻¹ IBA and 1.5 mg l⁻¹ BAP got elongated along with further proliferation of shoots. Repeated cycles of subculture of segments of 4-6 shoots gave elongated shoots concomitant with multiplication of shoots.A few shoots were rooted in the same medium but not all the shoots were rooted.
simultaneously in the multiplication medium. Hence individual shoots having 3-5 cm length were excised and transferred to the media containing 3mg/l IBA and 0.5mg/l BAP. The transferred shoots got rooted within 40 days of incubation in the rooting medium (Fig.43). The plantlets so obtained were hardened and transferred to the field by adopting the standard procedure.

3.3 SCREENING FOR SOMACLONAL VARIATIONS

The following observations were obtained from the study of morphological characteristics of in vitro regenerated and sucker derived nendran plants grown in the field. The shoot tip culture regenerated plants were transferred to the field, grown and studied for their first and second generations in the field. The growth characteristics of the plants were assessed taking into account their growth in length, and increase in girth at the base of the psuedostem.

3.3.1 SCREENING BY GROWTH AND YIELD CHARACTERS

In vitro shoot tip (STR) and inflorescence tip (ITR) culture regenerated plants and sucker grown (SG) plants were studied for growth and yield characters.

3.3.1.1 IN VITRO CULTURE REGENERATED NENDRAN PLANTS-

GROWTH CHARACTERS

(i) Height of the plants

(a) Plantlets were regenerated from Shoot tip explants cultured on medium containing MS1 nutrients supplemented with 30gl⁻¹ sucrose, 3mg/l BAP and 370mg/l NaH₂PO₄. The plants grew to an average height of 345.2cm. Such plants produced bunches within 12-13 months of growth and had normal bunches for the first generation. (Table 3.11 and Fig.44).
TABLE 3.11 COMPARISON OF PSUEDOSTEM HEIGHT OF NENDRAN

DERIVED FROM VARIOUS SOURCES.

<table>
<thead>
<tr>
<th>Source of seedlings</th>
<th>Average height in cm.</th>
<th>Month after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Shoot tip</td>
<td>81</td>
<td>111</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>66.8</td>
<td>98.0</td>
</tr>
<tr>
<td>Sucker grown</td>
<td>146.4</td>
<td><strong>208.5</strong></td>
</tr>
</tbody>
</table>

Significance at P-0.05 level

ANOVA

** JBF  P value 0.000

** MGP  P value 0.000

For more details see Appendix Table-A1


Based on pair wise comparison the following result is obtained

(a) ITR and STR

** JBF ITR and STR  P value 0.000

* MGP ITR and STR  P value 0.074

See table A-2

(b) ITR and SG

** JBF- ITR and SG  P value 0.000
**MGP- ITR and SG**  P value 0.000
See Table A-3

(c) STR and SG

**JBF- STR and SG**  P value 0.001

**MGP- STR and SG**  P value 0.000
See Table. A-4

The second generation plants took only 10 months for flowering. Shoot tip culture derived plants transferred to the field after the second subculture i.e. from third subculture onwards showed variations like dwarf plants (Fig. 45) and plants producing no flowers. Some plants grew to their normal size but did not produce flowers, instead dried off (Fig. 46). The dwarf and non-flowering plants repeated the same variations during the second generation also.

Apart from giving normal fruits and bunches the shoot tip culture derived plants showed an average height of 345.2 cm at the time of flowering (Fig. 47). Plants regenerated from shoot tip explants cultured on MS1 medium with 5 mg/l BAP and 370 mg/l sodium dihydrogen orthophosphate showed a range of variations. 50% plants grew to a height of 65-187 cms. But the plants produced no bunches, instead new suckers were produced from the plant leading to the degeneration and death of the mother plant (Fig. 48). 30.5% plants produced not even a single sucker, but the leaves turned yellow and slowly dried off leading to the death of the plant (Fig. 49). 19.5% plants matured and produced bunches having few slender fingers. Two of them were having long peduncle carrying small fruits (Fig. 50). The fingers were similar to the size of poovan fruits. These two bunches were having 34 and 36 fingers each and four hands.
(b) Inflorescence tip regenerated plants on MS1 medium containing 3 mgL⁻¹ BAP and 40 gl⁻¹ sucrose were transferred to the field and studied for their height. The data were collected at two months interval (Table 3.11). These plants showed an average height of 395.2 cm at maturity stage (Fig 51). The *in vitro* inflorescence tip regenerated plants took an average period of 16 months for maturation and flowering.

The *in vitro* regenerated plants showed an average height of 395.2 cm in comparison with the sucker grown plants, which showed an average height of 315.7 cm only (Fig 52). An interesting phenomenon observed is that in spite of such a height, i.e., a height of 395.2 cm the plants did not lodge and got destroyed. The second generation plants grown from suckers excised and isolated from the inflorescence apex culture regenerated mother plants also showed an average height 388 cm at maturity. Hence, the characters are similar to the tissue culture mother plants. At the same time the second generation plants produced flowers after 12 months of growth.

(c) The sucker grown plants produced bunches after 10 months of growth and shown an average height of 315.7 cm at the time of flowering (Table 3.11)

(ii). Girth of the plants.

(a) Shoot tip regenerated plants showed an average girth of 66.7 cm after 12-13 month of growth i.e., just before flowering (Table 3.12).
### Table 3.12: Comparison of Pseudostem Girth of Nendran Plants Derived from Various Sources.

<table>
<thead>
<tr>
<th>Source of seedlings</th>
<th>Average girth in cm</th>
<th>Month after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Shoot tip</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflorescence tip</td>
<td>19.3</td>
<td>26.3</td>
</tr>
<tr>
<td>Sucker grown</td>
<td>41.8</td>
<td><strong>59.8</strong></td>
</tr>
</tbody>
</table>

Significance at P -0.05 level

**ANOVA**

** JBF  P value 0.000

** MGP  P value 0.000

See table A-1

**Pair wise comparison**

(a) ITR and STR

** JBF  ITR and STR  P value 0.001

* MGP  ITR and STR  P value 0.833

See table A-2

(b) ITR and SG

* JBF- ITR and SG  P value 0.731

** MGP- ITR and SG  P value 0.000

See Table A-3

(c) STR and SG

** JBF- STR and SG  P value 0.001

** MGP- STR and SG  P value 0.000

See Table A-4
(b) The inflorescence tip regenerated plants showed an average girth of 80.2 cm after 16 months of growth, i.e., just before flowering (Table 3.12).

(c) The sucker derived plants showed an average girth of 78.2 cm at the time of flowering (Table 3.12)

(iii) **Yield characters of nendran**

The plants regenerated from shoot tip culture matured and produced flowers only after 12-13 months of planting, while the inflorescence apex regenerated plants took 15-16 months for flowering and fruit setting. 95.83% of the plants transferred to the field produced flowers, while 4.17% did not flower. These non-flowering plants grew to the size of 65 cm, and then ceased to grow concomitant with sucker production followed by the degeneration of the mother plant (Fig. 3). The normal plants produced normal flowers and fruits (Fig. 54). Among the flowering plants 4 normal plants were found to be inducing more suckers i.e. 8-10 suckers in comparison to sucker derived plants, which normally produce only 3-4 suckers (Fig. 55). In the case of normal plants derived from inflorescence apex culture there is observed a wide variation in the yield, namely 5.6 Kg to 14.8 Kg bunch weight. With regard to the inflorescence apex regenerated plants transferred to the field from the fourth subculture 18.18% plants were dwarf showing a growth of 90-180 cm in height (Fig. 56) Two plants grew to a size of 210-242 cm, but did not produce flowers and fruits, instead both plants dried off slowly (Figs. 57& 58, Table 3.13).
TABLE 3.13 - YIELD CHARACTERS OF IN VIVO AND IN VITRO REGENERATED NENDRAN PLANTS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regeneration from</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Inflorescence</td>
<td>Sucker</td>
<td></td>
</tr>
<tr>
<td>Average weight of bunches in Kg</td>
<td><strong>14.38</strong></td>
<td>10.38</td>
<td>13.75</td>
<td></td>
</tr>
<tr>
<td>Average number of hands</td>
<td>*6.1</td>
<td>5.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Average number of fingers</td>
<td><strong>59.60</strong></td>
<td>44.75</td>
<td>56.00</td>
<td></td>
</tr>
<tr>
<td>Time taken for flowering (months)</td>
<td>12</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Time taken for harvesting (months)</td>
<td>15</td>
<td>19</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Yield characters of Nendran

ANOVA

Between STR, ITR & SG

**No. of fingers** P value 0.000
*No. of hands** P value 0.053
**Bunch weight** P value 0.000

See Table A.5

Pair wise comparison

(1) STR & ITR

** No. of fingers P value 0.000
** Bunch weight P value 0.000

See Table A-6

(2) STR and SG

* No. of fingers P value 0.117
Suckers were collected from the field grown in vitro regenerated nendran plant and planted in the field to rear the second generation (Fig. 59). The second generation plants so grown were found to show the same morphological and yield characters similar to the first generation plants. Since the suckers were collected from the in vitro regenerated plants showing normal growth and yield characters, practically not much variations were observed in the second and third generations grown in the field. Similarly, in the variant dwarf plants the second generation plants showed all the characters similar to the mother plant.

3.3.1.2 IN VITRO CULTURE REGENERATED POOVAN PLANTS.

(i) GROWTH CHARACTERS

The plants appeared normal in growth similar with sucker derived plants. Plants regenerated from STR, ITR and SG of poovan demonstrated the following observations. Poovan shoot tip explants were regenerated from medium containing MS1 with 30 g l⁻¹ sucrose, 3 mg l⁻¹ BAP, 370 mg l⁻¹ NaH₂PO₄ and 160 mg l⁻¹ adenine sulphate.
(a) Shoot tip regenerated poovan plants-growth characters

*In vitro* shoot tip derived poovan plants shown an average height of 270.5 cm after 16 months of growth with an average girth of 91.3 cm at the base (Table 3.14 and 3.15).

**TABLE 3.14-COMPARISON OF PSEUDOSTEM HEIGHT OF POOVAN PLANTS DERIVED FROM VARIOUS SOURCES**

<table>
<thead>
<tr>
<th>Source of seedlings</th>
<th>Average height (cm)</th>
<th>Month after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot tip</td>
<td>33.1 59.5 108.3 169.1 215 246.3 270.5</td>
<td>4 6 8 10 12 14 16</td>
</tr>
<tr>
<td>Inflorescence tip</td>
<td>34.1 63.5 109.5 172.3 217.8 252.5 *276.3</td>
<td></td>
</tr>
<tr>
<td>Sucker derived</td>
<td>98.7 133.6 **195.2 232.3 254.3 272.1</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA

* JBF – Height P value 0.654
** MGP – Height P value 0.000

See Table A-9

(b) Poovan inflorescence tip-growth characters.

Similarly plants regenerated from inflorescence apex cultured on MS1 medium supplemented with 3mg/l BAP, 30g/l sucrose and 160mg/l adenine sulphate on transfer to the field gave the following observations regarding the growth of the plants. Here the average height observed at maturity stage was 276.3 cm and the average girth at the base of the plant is 92.1 cm (Table.3.14 and Table.3.15).
(c) Sucker derived -poovan

The sucker derived plants on field study and evaluation gave the following data.

The average height observed was 272.1cm at JBF and a girth of 91.3cm at JBF (Table 3.14 & Table 3.15).

**TABLE 3.15- COMPARISON OF PSEUDOSTEM Girth OF POOVAN PLANTS DERIVED FROM VARIOUS SOURCE**

<table>
<thead>
<tr>
<th>Source of seedlings</th>
<th>Average girth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Months after planting</td>
</tr>
<tr>
<td>Shoot tip</td>
<td></td>
</tr>
<tr>
<td>Inflorescence</td>
<td></td>
</tr>
<tr>
<td>Sucker derived</td>
<td></td>
</tr>
</tbody>
</table>

Here no significant difference between shoot tip and inflorescence tip regenerated plants at MGP stage. Similarly no significant difference between the three in girth measurements at JBF stage.

ANOVA

* JBF - girth  P-value 0.995

** MGP - girth  P-value 0.000

See Table A-9

Based on pairwise comparisons of the data of three parameters of poovan, the following results are obtained.

(1) Between STR and ITR

* MGP - Girth  P-value 0.96

* MGP - Height  P-value 0.740

See Table A-10
(2) Between STR and SG

* * MGP – Girth  P-Value 0.000
* * MGP – Height  P-Value 0.000

See Table A-11

(3) Between ITR and SG

* * MGP – Girth  P-Value 0.000
* * MGP – Height  P-Value 0.000

See Table A-12

(ii) Yield characters-poovan

The shoot tip derived plants flowered after 16 months of planting and produced fruits (Fig. 60). The bunch could be harvested after 3 months of flowering. Similarly the inflorescence apex derived plants also showed flowering and fruiting after 16 months of planting (Fig 61). The plants were normal in growth in the field. The bunches could be harvested after 3 months of fruit formation (Fig. 62 and Table 3.16)

**TABLE 3.16 - YIELD CHARACTERISTICS OF IN VIVO AND IN VITRO REGENERATED POOVAN PLANTS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Shoot tip</th>
<th>Inflorescence tip</th>
<th>Sucker derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weight of bunch in Kg</td>
<td>13.6</td>
<td><strong>14.4</strong></td>
<td>7.25</td>
</tr>
<tr>
<td>Average number of hands</td>
<td>5.7</td>
<td><strong>6.28</strong></td>
<td>4.5</td>
</tr>
<tr>
<td>Average number of fingers</td>
<td>68.8</td>
<td><strong>72.8</strong></td>
<td>64.7</td>
</tr>
<tr>
<td>Time for flowering (months)</td>
<td>16</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Time for harvesting (months)</td>
<td>19</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>
ANOVA

* No. of fingers  P value 0.208
** No. of hands  P value 0.002
** Bunch weight  P value 0.000

See Table A-13

**Based on pair wise comparison.**

1) Between STR and ITR

* No. of hands  P-value 0.1
* Bunch weight  P-value 0.102

See Table A-14

2) Between STR and SG

** No. of hands  P value 0.015
** Bunch weight  P value 0.000

See Table A-15

3) Between ITR and SG

** No. of hands  P value 0.005
** Bunch weight  P value 0.000.

See Table A-16

The mature bunches demonstrated same characteristics as that of sucker grown poovan plants in both types of *in vitro* regenerated plants.

The tissue culture poovan plants obtained from shoot tip as well as inflorescence tip culture were found to be infected by two diseases, panama wilt and bunchy top in the field. Out of the total plants planted in the college campus 4.10% plants among the shoot tip culture derived plants and 2.08% plants among
the inflorescence apex culture derived plants showed bunchy top disease (Fig 63). 33.33% plants planted in the campus of Adoration hostel showed infection of panama wilt disease (Fig 64). On the contrary, plants regenerated from inflorescence apex and shoot tip culture, planted in the campus of St. Thomas college did not show infections by panama wilt disease. It is also noted that, among the plants, regenerated from poovan inflorescence tip transferred to the field 2.08% demonstrated a variation of dwarfism. This plant grew only to a size of 75 cm in height, then induced 4 suckers. Gradually the mother plant showed yellowing of leaves and became dead. The 4 suckers produced did not even grow to a size of 75 cm, instead after some time of growth they also gradually degenerated and became dead. No such variations were observed in the case of shoot tip culture derived poovan plants. Out of the 48 inflorescence apex culture derived plants transferred and established in the field one plant showed the production of 8 suckers in comparison to normal sucker derived plants that produce only 4-5 suckers (Fig 66). Out of these eight suckers two of them grew to normal size that on transplanting attained maturity and produced bunches, while the others grew to a size of 75-122 cm, and then degenerated. Both types of tissue culture plants required an additional period of three more months for attaining maturity and flowering in comparison to the 13 months demonstrated by sucker derived plants.

3.3.2 SCREENING THE PHOTOSYNTHETIC EFFICIENCY AND METABOLITES.

An investigation was carried out to study the photosynthetically active pigments and oxygen evolution phenomena in the leaves of in vitro regenerated
nendran and poovan plants and the same was analysed in comparison with the control sucker grown plants.

3.3.2.1 CHLOROPHYLL CONTENT AND CAROTENOIDs

The tissue culture raised plants showed a high content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. The carotenoid content in sucker derived poovan plants is 647.5 μg/mg fresh weight of leaf but it is 112% more than in tissue culture raised poovan plants. In the case of nendran the tissue culture derived plants showed high carotenoid content than that of control plants, approximately 66.03% more than that of the control. But chlorophyll a, chlorophyll b and total chlorophyll content of the tissue culture raised plants were 22.6%, 11.3%, 18.9% less than that of the control plants (Table 3.17).

3.3.2.2 O₂ EVOLUTION

Oxygen evolution rate which is an indication of electron transport taking place in the chloroplast membrane of the leaves was same in poovan tissue culture raised plants as well as in control plants. But in the case of nendran the tissue culture plants showed a lower rate of oxygen evolution than that of the control (34 μmoles of oxygen/m²/minute) (Table 3.17).

3.3.2.3 TOTAL SUGAR

The total sugar content did not vary much between the poovan control and tissue culture derived plants. Whereas the total sugar content of tissue culture derived plants of nendran (123.59 μg/g fresh wt.) was more than that of the control (91.37 μg/g fresh wt.) (Table 3.17).
3.3.2.4 TOTAL FREE AMINO ACIDS

The total free amino acid content in tissue culture derived poovan (541.0 \( \mu \text{g/g fresh wt.} \)) was less than that of the control (662.5 \( \mu \text{g/g fresh wt.} \)). Whereas the total free amino acid content in tissue culture derived nendran (692.0 \( \mu \text{g/g fresh wt.} \)) was more than that of the control (585.0 \( \mu \text{g/g fresh weight} \)) (Table 3.17).

3.3.2.5 TOTAL PROTEINS

The study of total proteins has led to the conclusion that it is almost equal in the tissue culture derived plants and control plants of poovan and nendran. (Table 3.17).

### TABLE 3.17 - COMPARATIVE STUDY OF THE PHOTOSYNTHETIC FACTORS AND TOTAL METABOLITES IN IN VITRO AND IN VIVO PLANTS.

<table>
<thead>
<tr>
<th>Variants</th>
<th>Chl. a</th>
<th>Chl. b</th>
<th>Chl. a+b</th>
<th>Carotenoids</th>
<th>O2 evolution</th>
<th>Total free amino acids</th>
<th>Total sugars</th>
<th>Total proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poovan control</td>
<td>1962</td>
<td>839</td>
<td>2816</td>
<td>827.9</td>
<td>39</td>
<td>662.5</td>
<td>131.95</td>
<td>0.244</td>
</tr>
<tr>
<td>Poovan tissue culture</td>
<td>1637</td>
<td>705</td>
<td>2354</td>
<td>1587.8</td>
<td>39</td>
<td>541.0</td>
<td>130.0</td>
<td>0.244</td>
</tr>
<tr>
<td>Nendran control</td>
<td>1275</td>
<td>511</td>
<td>1796</td>
<td>647.5</td>
<td>45</td>
<td>585.0</td>
<td>91.37</td>
<td>0.24</td>
</tr>
<tr>
<td>Nendran tissue culture</td>
<td>1040</td>
<td>459</td>
<td>1508</td>
<td>1222.0</td>
<td>34</td>
<td>692.0</td>
<td>123.59</td>
<td>0.21</td>
</tr>
</tbody>
</table>

3.3.3 SCREENING FOR ISOENZYMES

3.3.3.1 TOTAL ACTIVITY OF ISOENZYMES

The isoenzyme activity of the leaf tissues collected from plants of nendran inflorescence tip culture, nendran shoot tip culture, nendran sucker derived plants...
and poovan inflorescence tip culture, shoot tip culture and sucker derived plants at two seasons and two stages of growth gave the following observations. Table 3.18 gives the isoenzyme activities at February-March period.

**TABLE 3.18- ISOENZYME ACTIVITY IN NENDRAN AND POOVAN BANANA PLANTS GROWN IN FEB-MARCH EXPRESSED IN UNITS/mg PROTEIN.**

<table>
<thead>
<tr>
<th>Variants</th>
<th>PAL</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nendran inflorescence tip culture derived</td>
<td>2.8</td>
<td>1.06</td>
<td>2240</td>
</tr>
<tr>
<td>Nendran shoot tip culture derived</td>
<td>0.5</td>
<td>1.62</td>
<td>3543</td>
</tr>
<tr>
<td>Nendran sucker derived</td>
<td>3.0</td>
<td>2.66</td>
<td>1319.5</td>
</tr>
<tr>
<td>Poovan inflorescence tip culture derived</td>
<td>2.05</td>
<td>1.11</td>
<td>857.1</td>
</tr>
<tr>
<td>Poovan shoot tip culture derived</td>
<td>1.431</td>
<td>1.91</td>
<td>830.0</td>
</tr>
<tr>
<td>Poovan sucker derived</td>
<td>6.0</td>
<td>2.76</td>
<td>208.0</td>
</tr>
</tbody>
</table>

The study of the isoenzyme activity from leaf tissue taken from 8-9 months old plants during the month of July- August gave the following observations. (Table 3.19).

**TABLE 3.19-ISOENZYME ACTIVITY IN NENDRAN AND POOVAN BANANA PLANTS GROWN IN AUGUST- SEPTEMBER, EXPRESSED IN UNITS/mg PROTEIN.**

<table>
<thead>
<tr>
<th>Variants</th>
<th>PAL</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nendran inflorescence tip culture derived</td>
<td>2.4</td>
<td>2.76</td>
<td>1417</td>
</tr>
<tr>
<td>Nendran shoot tip culture derived</td>
<td>0.7</td>
<td>2.125</td>
<td>2763</td>
</tr>
<tr>
<td>Nendran sucker derived</td>
<td>2.6</td>
<td>1.76</td>
<td>1612</td>
</tr>
<tr>
<td>Poovan inflorescence tip culture derived</td>
<td>2.73</td>
<td>1.61</td>
<td>987.5</td>
</tr>
<tr>
<td>Poovan shoot tip culture derived</td>
<td>1.24</td>
<td>2.062</td>
<td>920</td>
</tr>
<tr>
<td>Poovan sucker derived</td>
<td>4.2</td>
<td>2.68</td>
<td>842.6</td>
</tr>
</tbody>
</table>
In nendran and poovan plants PAL activity was greater in sucker derived plants compared to *in vitro* regenerated plants in both seasons and stages of growth. With regard to the activity of peroxidase enzyme, in nendran the inflorescence tip culture derived plants demonstrated maximum activity during later stages of growth in the August-September while in poovan greater activity was shown by the sucker derived plants in both seasons. In the study on the activity of PPO in nendran, the shoot tip derived plants showed the greater activity in comparison to inflorescence tip and sucker derived plants; while in nendran the PPO activity did not show much difference in all the three categories of plants during the growth season of July- August. Conversely, in the case of poovan the activity of PPO in sucker grown plants was reduced to one fourth in comparison to that of tissue culture plants irrespective of the type of explants used. (Tables: 3.18 & 3.19).

3.3.3.2. PARTIAL PURIFICATION AND DEVELOPMENT OF ELECTROPHORETIC PATTERN OF PPO

The PPO enzyme was subjected to partial purification study. The activity was expressed in units/gm tissue and demonstrated the activity of PPO during ammonium sulphate precipitation based on Warburg formulation chart. The precipitate was redissolved in extraction buffer and dialysed using a membrane for overnight and used for further assay. It was observed that the activity of PPO was significantly increased at 70% concentration.

The partially purified enzymes of these variants were subjected to NATIVE PAGE and incubated with substrate in order to reveal the isoenzyme pattern of PPO (Fig 67). The PPO activity of the leaves of nendran tissue culture
plants, nendran sucker derived plants, poovan sucker derived plants and poovan tissue culture derived plants showed four specific isoenzyme bands with Rf values 0.12, 0.20, 0.38 and 0.56 respectively. The intensity of enzyme reactions was almost the same in all the variants, but it was less in nendran tissue culture derived plants, which further confirms the reduction in activity (Fig. 67).

3.3.4. SUBCULTURE AND SOMACLONAL VARIATIONS

The multiple shoot obtained from shoot tip as well as inflorescence apex culture of nendran and poovan were cut into segments of 4-6 shoots and subcultured to the medium containing 3mg/l IBA and 1.5mg/l BAP. The shoots elongated concomitant with multiplication within a period of 60 days of incubation. The elongated shoots obtained on isolation and incubation on medium containing 3mg/l IBA and 0.5mg/l BAP got rooted within 45-60 days of transfer. Conversely, the smaller shoots obtained were cut into segments of 3-5 shoots and subcultured to medium containing 3mg/l IBA and 1.5mg/l BAP for elongation and multiplication of shoots. In each subculture the elongated shoots obtained were excised and transferred to rooting medium containing 3mg/l IBA and 0.5mg/l BAP for rooting, while, the smaller newly formed shoots were cut into segments of 3-5 and transferred to medium containing 3mg/l IBA and 1.5mg/l BAP for multiplication. This procedure was repeated for five consecutive subcultures. The rooted plants obtained in each generation is transferred to the field and screened for the presence of somaclonal variants. The interesting observation is that all the plants were similar in morphological characters at the time of transfer to the field. But once they were established in the field and grew, they demonstrated variations like, dwarfism, increase in the number of suckers produced, non-flowering etc. The
type of variants noticed in nendran shoot tip culture plants were mainly dwarf plants that did not grow to a normal size, nor produced flowers. In the case of nendran inflorescence tip culture, the variants observed were dwarf plants grown to a normal stature but did not produce flowers and flowering plants with enhanced number of suckers. The dwarf variants observed among the plants and plants obtained in each subculture were transferred and established in the field. The observations based on this study are given in Table 3.20. It was further observed that there is a gradual increase in the percentage of the dwarf shoots obtained as the number of subculture increases. The percentage of dwarf shoots was assessed after taking the number of dwarf plants among those transferred to the field from each cultures. In the case of shoots developed from nendran shoot tip culture also there is observed a corresponding increase in the percentage of dwarf shoots as the number of subculture increases namely from 48% in the first subculture to 76% in the 5th subculture.

**TABLE 3.20. PERCENTAGE OF OCCURRENCE OF DWARF SHOOTS AMONG PLANTS REGENERATED IN SUBCULTURE**

<table>
<thead>
<tr>
<th>Variants</th>
<th><strong>1st subculture</strong></th>
<th><strong>2nd subculture</strong></th>
<th><strong>3rd subculture</strong></th>
<th><strong>4th subculture</strong></th>
<th><strong>5th subculture</strong></th>
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<tr>
<td>Nendran shoot tip cultured in 3 mg l⁻¹ BAP</td>
<td>7</td>
<td>9</td>
<td>14</td>
<td>22</td>
<td>28</td>
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<tr>
<td>Nendran inflorescence tip culture</td>
<td>9</td>
<td>12</td>
<td>18</td>
<td>26</td>
<td>34</td>
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<tr>
<td>Poovan shoot tip culture</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>17</td>
<td>21</td>
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<tr>
<td>Poovan inflorescence tip culture</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>16</td>
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</tbody>
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ANOVA

* 1\textsuperscript{st} subculture  \hspace{1cm} P value 0.106
* 2\textsuperscript{nd} subculture  \hspace{1cm} P value 0.005
* 3\textsuperscript{rd} subculture  \hspace{1cm} P value 0.001
* 4\textsuperscript{th} subculture  \hspace{1cm} P value 0.000
* 5\textsuperscript{th} subculture  \hspace{1cm} P value 0.000.

(Appendix Table A-17 to A-21)

Apart from the dwarf plants, the frequency of non-flowering plants and the plants with enhanced number of suckers were also increased with the corresponding number of subcultures in nendran variety; whereas in the poovan variety non-flowering plants were not observed.

The type of variations observed in both poovan shoot tip and inflorescence tip culture were restricted to dwarf plants and plants showing increased number of suckers.

The dwarf shoots obtained in \textit{in vitro} culture of poovan and nendran were rooted in the rooting medium containing 3\textsuperscript{mg}l\textsuperscript{-1} IBA and 0.5\textsuperscript{mg}l\textsuperscript{-1} BAP and transferred to the field. In the case of nendran, the smaller dwarf shoots required an addition of 0.5\textsuperscript{mg}l\textsuperscript{-1} GA\textsubscript{3} for shoot elongation followed by rooting. These dwarf shoots on transfer to the field were found to grow to a size of 4-6\textsuperscript{ft} height only but never produced inflorescence or fruits.

A prominent morphological variation observed among the plants regenerated from cultured nendran inflorescence apex explants after 3\textsuperscript{rd} subculture was increased height of the plants concomitant with absence of lodging. The suckers were collected from such plants and planted in the field. The second and
third generation plants, so obtained demonstrated the same characteristic as the *in vitro* regenerated first generation plants.

An important advantage noticed in the poovan and nendran plants are that, practically none of the *in vitro* regenerated plants got affected by diseases like panama wilt, bunchy top etc. unless, the plants become infected from the soil, where the seedlings were planted. The plantlets are found disease free. Further, the *in vitro* inflorescence tip culture regenerated poovan plants were all high yielding.

Since the plantlets obtained in the multiplication medium and rooted in the rooting medium containing IBA and BAP demonstrated variation to a greater extent, an attempt was done using half strength liquid MS basal medium for rooting. It was observed that the plants rooted in the half strength liquid MS basal medium and transferred to the field showed practically no variation in the first and second generation shoot tip and inflorescence tip derived plants. At the same time it is to be noted that the dwarf plants did not induce rooting in half strength MS medium, rather they become rooted only in hormone containing medium.

### 3.3.5 ONTOGENY AND SOMACLONAL VARIATIONS

Study of the ontogenic characters of nendran and poovan in their meristematic regions revealed the following features.

#### 3.3.5.1 Nendran inflorescence

The nendran inflorescence tip incubated on culture medium initially contains meristematic regions in the axil of all the bracts. The meristems in the axil of lower 4-6 bracts grew and differentiate into one or more shoot bud meristem. In the process of differentiation the floral meristem revert back to vegetative meristem. On the contrary, the upper 4-5 bracts induce rudimentary flower
formation in the axil of bracts. Longitudinal section of explant after two months of culture showed induction of vegetative buds from the axil of lower bracts (Fig. 68). Prolonged incubation of inflorescence tip explants in the medium containing $3\text{mg} l^{-1}$ BAP induce cell division in some of the cells just above the cut end of the explant immediately in contact with the medium. These cells undergo division and organize into a small globular structures consisting of single epidermal layer enclosing small thin walled deeply staining cells with dense cytoplasm and prominent nucleus. The characteristics of the cells bring out the fact that they are physiologically active, and similar to normal meristematic cells (Fig. 69). Such an outgrowth at a later stage undergoes repeated division of the cells. These explants on transfer to a medium containing $1.5\text{mg} l^{-1}$ BAP and $0.5\text{mg} l^{-1}$ IBA differentiate vegetative buds from all over its surface.

The section of the floral apical meristem showing deep staining of cytoplasm of the cells clarify that the meristem is still in active stage. Longitudinal section of the explant after 70 days of culture showed that a single vegetative meristem differentiated from the axil of the lower bracts can give birth to two to three vegetative meristems by the process of adventitious budding (Fig. 68).

Eventhough the meristem existing at the tip of the explant is found to be active up to 70 days of incubation in the culture conditions, the explant tip became dried up within 90 days of culture concomitant with the development and growth of shoots from the axil of lower bracts.

The vegetative meristem developed from the axil of the bract has got the ability to proliferate into more than one bud in medium containing sufficient amount of hormones (Fig. 68).
3.3.5.2 Poovan inflorescence

Inflorescence tip of poovan in culture medium brings out induction of growth by certain *de novo* structures from the axil of the bracts. These growing structures were one or more globular protocorm like bodies, from which plants differentiate at a later stage.

Sections of poovan inflorescence tip explants incubated in culture conditions for twenty days demonstrated the growth of the bracts in the explants. On further incubation in the same medium from the axil of each lower bract there developed a single multicellular structure with a growing point. This structure without much differentiation simulates a protocorm (Fig. 70). As these structures grew further, they develop a regular epidermal layer, which turns wavy at certain locations (Fig. 71). Within the epidermal layer two types of cells are identified. The layer of cells inner to them consisting of vacuolated cells, towards the inner side with groups of procambial cells scattered here and there (Fig. 71). A few layers of cells immediately inner to the epidermal layer are composed of small cells with dense cytoplasm and nucleus. Moreover two or three growing spots consisting of 2-3 layers of cells having dense cytoplasm and prominent nucleus are noticed in the epidermal layer. These globular structures on further growth developed into a rather large structure with irregular outer epidermal layer giving the appearance of ridges and furrows on the outer surface of them (Fig. 72). On further growth, this protocorm like structure, become branched into three to four structures (Fig. 75), with irregular wavy epidermal layer (Fig. 73) showing the initiation of shoot meristem development (Fig. 74). Later meristems were differentiated from the outer surface of this body, each one of which develops into a shoot (Figs. 73 and
74). An interesting phenomenon noticed here is that new outgrowths developed from these globular protocorm like bodies from the peripheral layers of which also develops new meristems that gradually differentiate into shoot buds (Fig. 76). This pattern of bud formation observed here is clearly a case of adventitious budding.

Apart from the axil of the lower most 3 or 4 bracts, from the axil of all other upper bracts, rudimental male flowers alone develop. Finally, the inflorescence tip meristem ends up in the formation of 4 to 6 flowers (Fig. 77). At later stage ie., after 90-110 days of culture the apex of the explant dry up along with the differentiation and growth of buds from the protocorm like bodies.

3.3.5.3 Shoot tip of nendran

In nendran shoot tip the existence of meristem is already reported at the leaf- opposed location of the shoot bud. Each meristem existing in the leaf- opposed position undergoes division and differentiates into a single shoot. This phenomenon is quite similar to that of sucker formation in nature shown by nendran banana plants. This pattern of response is shown in the medium containing 3mg/l \(^{-1}\) BAP. But the pattern of response differs in medium containing higher concentrations of BAP like 5 or 10mg/l \(^{-1}\). In this medium 2 or 3 corm like structures grew out from the base of the explant and developed. Longitudinal sections of these corm like structures show a very compact arrangement of cells delimited by an outer epidermis (Fig. 78). 3 to 5 layers inner to the epidermis consists of compactly arranged deeply staining small cells showing that they are physiologically highly active. On the contrary, the cells of the central layers are larger and vacuolated. These corm like structures on transfer to 0.5mg/l \(^{-1}\) IBA and
3mgL⁻¹ BAP differentiate shoots from all over its surface, resulting in the production of an average number of 15 shoots from one corm-like structure.

3.3.5.4 Shoot tip of poovan

Longitudinal sections of excised shoot tips of poovan brought from the culture conditions are observed for the study. After 60 days of culture the explant showed the development of one or more protocorm-like bodies from the leaf opposed position of the shoot tip explant (Fig. 79). Initially these structures show an elliptical shape with one or two growing points. Such structures from the leaf opposed position of 3-4 lower leaf sheathes, on further incubation in the culture medium, grew and developed. Longitudinal section of these protocorm-like bodies after 70 days of culture demonstrated an anatomical structure similar to the one developed from cultured inflorescence apex; namely an outer one layered epidermis, inner few layers of small thin walled deeply stained cells and an innermost zone of large vacuolated cells (Fig. 80). These bodies at a later stage on longitudinal sectioning found to differentiate several meristems from its outer layers (Fig. 81). Each such meristem on further incubation organized as a shoot. Hence the explants of poovan shoot tip cultures show clusters of shoots aggregated together into 3 or 4 groups, each group consisting of 4 to 6 shoots developed from a single protocorm-like body.
3.3.6 BIOFERTILIZER APPLICATION

3.3.6.1 The shoot tip regenerated nendran plants

(i) Growth characters

The T1 treated plants had an average height of 209 cm with an average girth of 46.3 cm. T2 plants showed an average height of 291 cm and 56 cm girth just before flowering. The T3 and T4 plants showed an average height of 277 and 218 cm respectively, with a girth of 49 and 44 cm each. The data are collected at two months intervals, starting from 4 months after planting in the field (Fig. 82).

3.3.6 Nendran-shoot tip culture regenerated plants

(ii) Yield characters

The study of the yield characteristics gave the following result. The T2 plants had an average number of 55.2 fingers in 5 hands with a total average weight of 16.3 Kg. All other treatments showed only a lower yield (Fig. 83).

3.3.6.2 Nendran plant regenerated from inflorescence tip culture

(i) Growth characters

The inflorescence apex regenerated plants treated with *azospirillum* and NPK fertilizer in various combinations demonstrated the results given below. The plants treated with T2 combination had an average height of 344.8 cm with an average girth of 64.2 cm just before flowering. On the contrary the T1, T3 and T4 plants showed an average height of 249.3 cm, 283 cm and 272 cm and an average girth of 54.2 cm, 63.6 cm and 60.5 cm respectively (Fig. 84).

3.3.6.2. Nendran inflorescence tip regenerated plants

(ii) Yield characters
Regarding the yield characters of nendran inflorescence derived plants the T2 plants gave an average number of 57 fingers in 6 hands with a total average weight of 16.5 Kg. The yield characteristics shown by T1, T3 and T4 plants were lower than the T2 plants (Fig. 85).

3.3.6.3 Sucker derived nendran

(i) Growth characters

The field study of sucker derived nendran plants gave the following results. The T2 plants had an average height of 312 cm and girth of 78.5 cm. On the contrary the T1, T3 and T4 plants had only an average height of 249.2 cm, 283 cm and 272.5 cm and an average girth of 66.7 cm, 63.6 cm and 60.5 cm respectively (Fig. 86).

3.3.6.3 Sucker derived nendran

(ii) Yield characters

The average yield given by the T2 plants was 18.3 Kg with 55.2 fingers in 5.2 bunches. Conversely all other treatments had an yield characteristic lower than the T2 treatment (Fig. 87).

3.3.6.4 Shoot tip culture derived poovan plants

(i) Growth characters

The T2 plants were found to grow a height of 230.6 cm with an average girth of 66.1 cm. The T1 plants had an average girth of 59 cm just before flowering. The T3 plants showed an average height of 226.3 cm and an average girth of 56.4 cm in comparison with T4 plants that showed an average height of 204 cm and an average girth of 56.9 cm just before flowering (Fig. 88).
3.3.6.5 Shoot tip culture derived poovan plants

(ii) Yield characters

With regard to yield characters the T2 plants produced bunches having an average weight of 11 Kg with 76 fingers arranged in 6 hands. On the other hand the T1, T3 and T4 bunches had an average weight of 7.1 Kg, 10.5 Kg and 7.5 Kg respectively (Fig.89).

3.3.6.5. Inflorescence tip regenerated plants – poovan

(i) Growth characters

The inflorescence tip regenerated plants treated with *azospirillum* and NPK fertilizer in various combinations demonstrated the following results. The plants treated with T2 combination had an average height of 288 cm and with an average girth of 54.8 cm just before flowering. But the other treatments such as T1, T3, T4 and control plants showed an average height of 224 cm, 246 cm, 238 cm, and 169 cm respectively (Fig.90).

3.3.6.5. Inflorescence tip regenerated plants

(ii) Yield characters

In inflorescence tip regenerated plants, the yield responses showed that the T2 plants gave the maximum bunch weight, i.e., 10.250 Kg with 75 fingers arranged in 7 hands. The other treatments like T1, T3, T4 and control showed a bunch weight of 7.5 Kg, 8.150 Kg, 7.125 Kg and 6.500 Kg respectively (Fig.91).

3.3.6.6 Poovan sucker derived plants

(i) Growth characters

In this treatment the T2 plants exhibited an average height of 288.6 cm. The other treatments as T1, T3 and T4 shown an average height of 220.3 cm, 274.1 cm
and 233.0 cm with an average girth of 65.6 cm, 76.6 cm and 71.6 cm respectively just before flowering (Fig. 92).

3.3.6.6. Poovan sucker derived plants

(ii) Yield characters

Yield response of sucker derived poovan plants, the T2 treatment obtained a bunch weight of 10.7 Kg with 82 fingers with an average of 5.2 hands. The T1, T3 and T4 treatments had a bunch weight of 6.5 Kg, 6.6 Kg and 6.7 Kg with an average number of 58.5, 63.1 and 69 fingers respectively (Fig. 93).
Fig. 1. Responding nendran inflorescence tip explants in MS medium containing 3 mgl\(^{-1}\) BAP after 120 days of culture showing 8 buds.

Fig. 2. Responding inflorescence explant on MSI medium with 3mgl\(^{-1}\) BAP and 3Ogl\(^{-1}\) sucrose after 30 days of culture showing initiation of growth response.

Fig. 3. Same as in Fig. 2 after 60 days of culture showing 4 buds.

Fig. 4. Same as above after 90 days of incubation showing 8 buds.

Fig. 5. Same as above after 120 days in culture medium showing 15 buds.

Fig. 6. Responding explant on MSI medium with 3mgl\(^{-1}\) BAP and 40gl\(^{-1}\) sucrose after 30 days of culture showing initiation of growth.

Fig. 7. Same as in Fig. 6, showing 8 buds after 60 days of culture.

Fig. 8. Same as in Fig. 7, after 90 days of culture showing 14 buds.
Fig. 9. Same as in Fig. 7, showing a later stage after 120 days of culture demonstrating 18 buds.

Fig. 10. A cluster of elongated shoots on MSI medium, with 3 mg/l IBA and 1.5 mg/l BAP after 45 days of subculture.

Fig. 11. An excised shoot transferred to MSI medium with 3 mg/l IBA and 0.5 mg/l BAP for rooting.

Fig. 12. An elongated shoot on rooting medium with 3 mg/l IBA and 0.5 mg/l BAP showing 3 roots with root hairs.

Fig. 13. Inflorescence tip regenerated plantlet after four weeks of transfer to plastic cups.

Fig. 14. Inflorescence tip regenerated nendran plants established in the field after 45 days of transfer to the field.
Fig. 15. Nendran inflorescence tip explants cultured on MS1 medium containing 40 g l$^{-1}$ sucrose and 5 mg l$^{-1}$ BAP showing 38 buds after 120 days of culture.

Fig. 16. Shoots induced in MSI medium containing 5 mg l$^{-1}$ BAP and 40 g l$^{-1}$ sucrose, elongated in medium supplemented with 0.5 mg l$^{-1}$ GA3 and rooting initiated in medium containing 3 mg l$^{-1}$ IBA and 0.5 mg l$^{-1}$ BAP.

Fig. 17. Responding explants of poovan inflorescence tip in MS medium with 3 mg l$^{-1}$ BAP showing flower formation in the axil of bracts.

Fig. 18. Explant in MSI medium with 3 mg l$^{-1}$ BAP and 30 g l$^{-1}$ sucrose showing growth of flower buds and shoot after 120 days of culture.

Fig. 19. Responding poovan inflorescence tip explants in MSI medium and 100 mg l$^{-1}$ adenine sulphate showing flower formation, followed by induction of shoots after 90 days of culture.

Fig. 20. Same as in Fig. 19 after 120 days of culture showing further shoot initiation and degenerating flower.

Fig. 21. Responding explant in MSI medium with 3 mg l$^{-1}$ BAP and 160 mg l$^{-1}$ adenine sulphate showing active growth after 30 days of culture.

Fig. 22. Explants showing special pattern of response after 60 days of culture showing growth of three protocorm like bodies from the explant.
Fig. 23. Same as in Fig. 22 showing the protocorm like bodies with meristem emerging out from them.

Fig. 24. Responding explant with groups of shoots developed from protocorm like bodies.

Fig. 25. The responding explant with 32 buds after 150 days of culture.

Fig. 26. Cluster of shoot buds transferred to MSI medium containing $3\text{mg}^{-1}$ IBA and $1.5\text{mg}^{-1}$ BAP showing shoot multiplication concomitant with shoot elongation after 60 days of culture.

Fig. 27. An isolated shoot on rooting medium containing $3\text{mg}^{-1}$ IBA and $0.5\text{mg}^{-1}$ BAP with roots and root hairs after 45 days of transfer to rooting medium.

Fig. 28. Rooted plant transplanted to the field from greenhouse condition and established in the field.

Fig. 29. Poovan inflorescence tip explants cultured on MSI medium with $3\text{mg}^{-1}$ BAP, $160\text{mg}^{-1}$ adenine sulphate and $370\text{mg}^{-1}$ sodium di hydrogen orthophosphate showing induction of 4 buds with a degenerated explant tip.

Fig. 30. Responding shoot tip explant of nendran with corm like structure initiated in MS1 medium with $5\text{mg}^{-1}$ BAP and $370\text{mg}^{-1}$ NaH$_2$PO$_4$ transferred to medium supplemented with $0.5\text{mg}^{-1}$ IBA and $3\text{mg}^{-1}$ BAP.
Fig. 31. Corm like structures excised from responding explants developed in Fig. 30 and transferred to medium containing 0.5mg l<sup>-1</sup> IBA and 3mg l<sup>-1</sup> BAP showing induction of small buds after 120 days of transfer.

Fig. 32. Dwarf shoots produced on corm induced in MSI medium with 5 mg l<sup>-1</sup> BAP and 370mg l<sup>-1</sup> NaH<sub>2</sub>P<sub>O</sub><sub>4</sub> after 150 days of culture to medium containing 0.5mg l<sup>-1</sup> IBA and 3mg l<sup>-1</sup> BAP. Note the thick fleshy leaves of the plant.

Fig. 33. Responding nendran shoot tip explant with multiple shoot in MS1 medium containing 3mg l<sup>-1</sup> BAP and 370mg l<sup>-1</sup> NaH<sub>2</sub>P<sub>O</sub><sub>4</sub> after 120 days of culture.

Fig. 34. Responding poovan shoot tip explants on MS medium containing 3mg l<sup>-1</sup> BAP showing single shoot formation.

Fig. 35. Explant cultured on MS1 medium with 3mg l<sup>-1</sup> BAP after 120 days of culture showing multiple shoot formation.

Fig. 36. Responding explants cultured on MS1 medium with 5mg l<sup>-1</sup> BAP after 120 days of culture showing dwarf shoots.
Fig. 37. Responding poovan shoot tip explant cultured on MS1 medium supplemented with 370\(\text{mg}\,\text{l}^{-1}\) \(\text{NaH}_{2}\text{PO}_4\) 160\(\text{mg}\,\text{l}^{-1}\) adenine sulphate and 3\(\text{mg}\,\text{l}^{-1}\) BAP after 30 days of culture.

Fig. 38. Responding explants in medium containing MS1 nutrients with 370\(\text{mg}\,\text{l}^{-1}\) \(\text{NaH}_{2}\text{PO}_4\) and 160\(\text{mg}\,\text{l}^{-1}\) adenine sulphate showing whitish globular outgrowth. — showing globular growth.

Fig. 39. Responding shoot tip explant showing developed greenish protocorm like body.

Fig. 40. Responding explant in Fig. 39 at a later stage of development showing cluster of shoots developed from a protocorm like outgrowth.

Fig. 41. Same as in fig. 40 after 90 days of culture showing three groups of shoots developed from the explant.

Fig. 42. Same as in fig. 40 after 120 days of culture showing cluster of shoots emerged from each protocorm like bodies.

Fig. 43. Poovan shoot tip derived plant rooted in MS1 medium with 3\(\text{mg}\,\text{l}^{-1}\) IBA and 0.5\(\text{mg}\,\text{l}^{-1}\) BAP.

Fig. 44. Mature nendran plant with bunch regenerated from shoot tip explant incubated on MS1 medium containing 370\(\text{mg}\,\text{l}^{-1}\) \(\text{NaH}_{2}\text{PO}_4\) and 3\(\text{mg}\,\text{l}^{-1}\) BAP.
Fig. 45. Shoot tip culture derived nendran plant transferred to the field after the 4th subculture showing variations like dwarfism and degeneration.

Fig. 46. Shoot tip culture derived plant in the field after 12 months of growth instead of producing flowers showing degeneration after attaining normal growth.

Fig. 47. Shoot tip culture derived normal nendran plant showing an average height of 345.2cm.

Fig. 48. Nendran shoot tip culture derived dwarf plant regenerated in MS1 medium with 5mg l⁻¹ BA and 370mg l⁻¹ NaH₂PO₄ after 10 months of growth in the field. (Note the suckers at the base of the plant).

Fig. 49. Nendran shoot tip culture regenerated plant same as above after 13 months of growth, without suckers and with degenerated leaves.
Fig. 50. A variant plant with bunch having long peduncle and small fruits.

Fig. 51. Plants regenerated from Nendran inflorescence tip transferred, established and growing in the field.

Fig. 52. Inflorescence tip culture derived normal nendran plant with a maturing bunch showing a height of 395.2 cm.

Fig 53. A gradually degenerating inflorescence tip culture derived nendran plant showing 65 cm height with suckers.

Fig 54. Normal inflorescence tip culture derived nendran plant with flowers and fruits.

Fig. 55. A variant, inflorescence tip culture derived mature nendran plant showing 8 suckers.
Fig. 56. A variant plant regenerated from inflorescence tip culture. The plant is regenerated in 4th subculture and showing only a height of 122cm.

Fig. 57. *In vitro* regenerated plant normal in growth pattern but no flowering and bunch production.

Fig. 58. *In vitro* inflorescence tip culture regenerated plant without producing a bunch having height of 242 cm showing degeneration.
Fig 59. Second and third generation plants obtained by planting suckers of *in vitro* regenerated plants grown in the field.

Fig. 60. Shoot tip culture derived poovan plant after 16 month of planting.

Fig. 61. Maturing bunch produced on inflorescence tip culture regenerated poovan plants.
Fig 62. A mature bunch of *in vitro* inflorescence apex regenerated poovan plant showing hands and fingers.

Fig. 63. *In vitro* Inflorescence tip culture derived poovan plant demonstrating bunchy top disease symptoms.

Fig 64. *In vitro* regenerated poovan plant in the field, after 5 months growth, showing panama wilt disease symptoms.

Fig. 65. A dwarf *in vitro* regenerated poovan plant having 75 cm. Height with degenerating leaves.

Fig. 66. Variant poovan inflorescence apex culture regenerated plant that produced 8 suckers.
Fig. 67. Isoenzyme pattern of polyphenol oxidase (PPO) in the third leaf of nendran and poovan banana plant. 1, 2, 3 and 4 represent the isoenzyme bands. NT. Nendran inflorescence tip culture. NS. Nendran sucker derived. PT. Poovan inflorescence tip culture. PS. Poovan sucker derived.

Fig. 68. L.S. of the nendran inflorescence tip after two months of culture, showing the induction of vegetative buds from the axil of lower bracts. Note the adventitious meristem formed in the axil of the bracts.

Fig. 69. Longitudinal section of a group of meristematic cells formed, in to a globular structure surrounded by a single layered epidermis. The cells are deeply staining with dense cytoplasm and prominent nucleus showing typical characteristics of meristematic cells.

Fig. 70. L.S. of the axil of one bract of the cultured poovan inflorescence tip showing developing protocorm like body with elliptical shape.

Fig. 71. Protocorm like body, at a later stage showing outer regular peripheral layer turning irregular at the growing tip. Note the single layered epidermis and the growing points at the tip of the body.

Fig. 72. Protocorm like structure at a later stage of growth, developing ridges and furrows. The structure is seen attached at the axil of the bract of the inflorescence explant.

Fig. 73. A portion of the protocorm like structure showing development of large number of meristems from the surface of the structure.

Fig. 74. A single meristem differentiated from protocorm like body.
Fig. 75. Diagramatic representation of the L.S. of the responding inflorescence tip explant with two structures at a later stage of development in the axil of the bracts. From the surface of such structure large number of meristems are found developing.
Fig. 76. A branch developed from one of the protocorm like body developed from poovan inflorescence tip showing differentiating meristems (←).

Fig. 77. L.S. of the apex of the inflorescence tip of poovan showing 6 rudimental male flowers developed.

Fig. 78. L.S of corm like structure developed in nendran shoot tip cultured in 5mg/l BAP showing a very compact arrangement of outer layers of cells delimited by an outer epidermis. Note one fully developed shoot meristem, the corm and two groups of cells in initiating meristem (←).

Fig. 79. Responding poovan shoot tip explant showing degenerating shoot tip and the developed protocorm like body (←).

Fig. 80. L.S. of the responding pcovan shoot tip explant with the developed protocorm like body. The protocorm like body is having growing points and developing meristems (←).

Fig. 81. A portion of L.S of protocorm like body in Fig. 80 showing two developed adventitious buds (←).
Fig. 82. GROWTH CHARACTERS OF SHOOT TIP CULTURE REGENERATED NENDRAN PLANTS CMS

Fig. 83. SHOOT TIP DERIVED NENDRAN PLANTS – YIELD CHARACTERS
Fig. 84. GROWTH CHARACTERS OF NENDRAN INFLORESCENCE TIP CULTURE REGENERATED PLANTS IN CMS

Fig. 85. NENDRAN INFLORESCENCE TIP REGENERATED PLANTS YIELD CHARACTERS
Fig. 88. SHOOT TIP CULTURE DERIVED POOVAN – GROWTH CHARACTERS IN CM

Fig. 89. POOVAN SHOOT TIP DERIVED PLANTS - YIELD CHARACTERS
Fig. 92. POOVAN SUCKER DERIVED PLANTS GROWTH CHARACTERS IN CM

Fig. 93. SUCKER DERIVED POOVAN – YIELD CHARACTERS