In vitro cloning and homestead cultivation of primitive Musa cultivars

S Mukunthakumar & S Seeni*

Plant Biotechnology Division, Tropical Botanic Garden and Research Institute, Pacha Palode, Karimancode P.O., Thiruvananthapuram 695 562, India.

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Two primitive diploid Musa cultivars, Matti and Chemmatti from the extreme southern part of the Western Ghats were multiplied by in vitro culture of sucker-derived shoot apices. Decontaminated corm explants (1 cm × 1 cm) having shoot apex (~ 0.3 cm) cultured for 1 month in Murashige and Skoog basal agar medium was cut vertically into eight segments and each segment having a part of shoot meristem was cultured in presence of 6-benzylaminopurine (BAP) and combinations of BAP and indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) to produce multiple shoots. After 12 weeks of culture, maximum number of shoots (32) in both the cultivars were produced in approximate 60% of the explants in presence of BAP and IAA each at 1.5 mg/1 (Matti) and 40% of the explants in 2.5 mg/1 of BAP and 1.5 mg/1 of IAA (Chemmatti). Buds were formed from the base of the subcultured shoots and somewhat more number (34) of shoots were obtained in Matti than in Chemmatti (31) after 8 weeks. Difference in the concentration of cytokinin required for shoot initiation and multiplication, persistence of exudation through the subculture and red colouration of the early formed sheathing leaf bases in the shoots in Chemmatti indicated possible genotypic differences between the two cultivars. Multiple shoot proliferation achieved through five subcultures of the isolated shoots without any decline. Transfer of shoots (4-5 cm) into MS basal medium favoured rooting in 4 weeks and rooted plants (9 cm) were hardened and established (80-95%). Mericlones of Matti cultivated in homesteads produced bunches of uniform characters in 13 months.

Keywords: Chemmatti, Homestead cultivation, Matti, Micropropagation, Musa, Native diploids

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Bananas belonging to the genus Musa are the most important of the tropical fruits, and a major tropical food crop with annual world production of around 40 million tones. Although, most of the bananas are sterile, propagated essentially by vegetative means, they have become genetically diverse largely through the accumulation of somatic mutations. As such, most of the domesticated and cultivated varieties are developed over several hundred years of evolution through somatic mutation, parthenocarpy, sterility and polyplody. Our dependence on only a few selected high yielding clones for cultivation has resulted in serious erosion of genetic diversity within the crop and repeated cloning has also rendered it susceptible to many diseases. Banana is widely recognized as the most depleted of all the agricultural crops. Certain selections of landraces are cultivated in small scale by the rural farmers in narrow pockets close to the areas of their natural distribution while there are many other lesser-known taxa yet to be documented and characterized. The area as a whole is under overwhelming biotic pressure, necessitating prudent conservation practices for those that still remain in nature and under poor farming conditions in remote areas.

Matti and Chemmatti (Sannachenkadali) are two of the rare primitive cultivars of diploid AA genome constitution exclusively cultivated in backyard farms and home gardens of villages adjoining to Agasthiyamalai forests in Kanyakumari district of southern Tamilnadu. Both of them take about...
15 months to mature and the ripe fruits are of high quality with sweet flavour. Matti otherwise called Dhevankadali is characterized by production of heavy bunches (7-15 kg.) bearing golden yellow fruits, less hard and occasional seeding. Kaani tribes of the southern Western Ghats make use of the corm extracts of Matti for jaundice\(^1\). Chemmatti is a hardy clone producing smaller bunches (7-10 kg.) with red to orange-red fruits. Hairy peduncle is characteristic of the clone and unlike Matti, the ripe fruits drop off easily from the pedicel. As part of our effort to conserve and popularize economic species of the southern ranges of the Western Ghats, this work was undertaken to achieve rapid \textit{in vitro} multiplication of Matti and Chemmatti and evaluation of the plants so multiplied through homestead cultivation. Our long-term objective is to make use of the desirable attributes of the landraces and primitive cultivars for improvement of the widely cultivated varieties through biotechnological means\(^1\).

Materials and Methods

Suckers (approx. 18 cm diam. and 35 cm long.) of Matti and Chemmatti were collected from local farms at Pechiparai village in Kanyakumari, India and brought to the laboratory for further processing. After defoliation and removal of the roots, the pseudostems (30 cm long) were washed well in running tap water to remove soil particles. The sheathing leaf bases were removed one by one and the pseudostem trimmed to approx. 10 cm long and 5 cm diam. They were then washed well in 2% (v/v) labolene (Godrej, India) detergent, surface sterilized by immersion for 20 min in 5% sterilil (Combichem, Delhi) and rinsed 3 to 4 times in sterile distilled water. The primordial leaves were then removed aseptically until the shoot apex of 0.3 cm size was exposed. Without damaging the shoot apex the surrounding corm tissue was trimmed to 1x1 cm size and implanted vertically into Murashige and Skoog\(^9\) basal agar nutrient medium containing macro salts at full, half and quarter strength micronutrients and vitamins, 3% (w/v) of sucrose and 0.6% of agar (CDH, New Delhi). In order to induce bud breaking different cytokinins viz., 6-benzyl aminopurine (BAP), kinetin (Kn), 2-isopentenyladenine (2-ip) were tested individually at concentrations of 1-5 mg/l\(^1\) and in combination with 1-2 mg/l\(^1\) of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA). All plant growth regulators were procured from Sigma Chemical Co., USA. The pH of the medium was adjusted to 5.6 before adding agar and autoclaved at 121°C under 104 kPa for 18 min. The cultures were incubated in a culture room maintained at 25°±2°C under an illumination of 30-50 \(\mu M^2 \cdot s^{-1}\) at 12 hr photoperiod.

After one month of culture in the basal medium, the swollen shoot apex with the surrounding corm tissue was cut vertically into eight segments and each segment having a part of shoot meristem was transferred to medium containing individual cytokinins and combination of a cytokinin and auxin. Due to limited number of suckers available for the experiments, each treatment at a time consisted of only four segments and was repeated thrice. After 12 weeks of culture, multiple shoot/buds proliferated upon each segment were separated and subcultured for 8 weeks in medium containing a combination of 1.5 mg/l\(^1\), BAP and 1.5 mg/l\(^1\), IAA (Matti) and 2.5 mg/l\(^1\), BAP and 1.5 mg/l\(^1\), IAA (Chemmatti) for further multiplication. After 3-5 subculture passages each of 8 week duration, individual shoots of 4-5 cm length were separated and transferred to MS basal medium for 4 weeks to induce shoot elongation and root formation. Statistical analysis was performed on the results of each experiment with the software SPSS/PC Version 4.0 (SPSS Inc., Chicago, USA) and the data were compared using ANOVA and LSD multiple range test.

Rooted plants were separated washed in tap water and transplanted in 20x13 cm polythene bags filled with potting mixture (1:1:1) of river sand, farmyard manure and topsoil. The plants were hardened in a mist chamber maintained at 70-80% RH for 3-4 weeks and subsequently maintained in the nursery for 4 weeks before distribution to interested individuals for homestead cultivation in Thiruvananthapuram and Kollam districts of Kerala. After 12-15 months, data on field performance were collected from the individuals and statistically analyzed.

Results and Discussion

Under the conditions of explant preparation and surface decontamination nearly 95% of the explants from both the cultivars were free from infection after 4 weeks of culture in MS basal medium. During the first 3 weeks the explants swelled up to 1.5 cm in diam. even while a marginal increase in height (0.7 cm) and greening of the outer leaf sheath surrounding the shoot apex were noticed. Somewhat increased growth response was noticed when
macrosalts were used at half strength. The cut surface of the explants turned to light or dark brown in all the cases. The lateral and longitudinal growth of the explants as evidenced from swelling ceased after 4 weeks (Fig. 1d), which probably indicated that endogenous hormones were insufficient to induce further growth. Exogenous application of PGRs became obligatory to induce differentiation of shoots in the explants. The explants cut vertically into eight segments and transferred into medium containing various concentrations of cytokinins and combinations of cytokinin and auxin in Chemmatti responded rather rapidly as reported in other Musa cultivars. The number of shoots formed in different concentrations of individual cytokinin varied from 2 to 10. On average, after 12 weeks of culture, BAP induced the formation of more number (10) of shoots than Kn (6) and 2iP (4). The preference of BAP having strong cytokinin activity over other cytokinins is reported in different cultivars of banana, other monocotyledons, and dicotyledons. Both the cultivars responded alike to treatments of individual concentrations of cytokinin and no difference could be made out between them in any of the type and concentration of the cytokinins tested.

Although, exogenous supply of cytokinin was essential and sufficient to induce multiple shoot formation, the response was greatly enhanced by using BAP in combination with auxins (IAA, IBA). Multiple shoot formation occurred in all the combinations tested and the number of shoots formed were more than individual concentrations of BAP could induce. The synergistic action of cytokinin and auxin in inducing multiple shoot formation is well established for other diploid and triploid cultivars of Musa as well. After twelve weeks of culture, maximum number of shoot formation was recorded in 60% of the segments in Matti and 40% in Chemmatti. In Matti maximum shoots proliferated in a combination of BAP and IAA each at 1.5 mg/1 closely followed by a combination of BAP and IBA of the same concentration (Fig. 2A, B). Besides, morphogenetic responses of the segments in Matti as a whole were better in presence of IBA than those of Chemmatti (Fig. 2D). However, the observation that higher concentration of BAP (2.5 mg/1) in combination with IAA (1.5 mg/1) was needed for optimal shoot formation in Chemmatti (Fig. 2C) indicated difference in cytokinin concentration requirement, which could be related to possible genotypic differences between the two cultivars. More or less same number shoots (32) were formed at the optimal combination of BAP and IAA or IBA in both the cultivars (Fig. 1e, f). This was probably related to diploid AA genome constitution of both the cultivars. However, fewer shoots are reported to be produced by cultivars of AAB compared to AAA composition. The remarkable increase in shoot morphogenesis particularly when the concentration of any of the two auxin types was raised from 1 mg/l to 1.5 mg/l was unparalleled in the published reports on in vitro multiplication of Musa cultivars. This is despite the fact that both the diploid cultivars showed maximum number of shoot formation in BAP/IAA than in BAP/IBA combination. The results also indicated that shoot height was not always inversely correlated with shoot number as the mean height recorded with optimal combinations involving IAA and BAP at 1.5 mg/l each in Matti was more than that obtained with other concentrations of the auxin tested. It was also noticed that the reduction in shoot number in elevated level (2 mg/l) of auxin (IBA) was particularly drastic in Chemmatti and this reduction was not paralleled by a similar increase in shoot height.

Multiplication of the shoots was achieved by subculturing individual shoots and shoot buds into the same media optimized for shoot initiation. The number of callus-free shoots regenerated from the base of the shoot buds presumably due to axillary meristem proliferation varied between the two cultivars, yet again confirming the genotypic influence on morphogenetic expression. Although, percentage response was nearly 100% in both the cultivars, somewhat more number of shoots (34) were obtained in Matti than in Chemmatti (31). Besides, exudation persisted particularly through the subculture of Chemmatti, which may account for inhibition and consequent less number of shoot formation. The robust nature of the rhizomatous base of the shoots and red colouration of the early-formed sheathing leaf base (Fig. 1f) in Chemmatti were other differences observed. These results suggested that though, they were of the same AA genomic composition, the two cultivars could be differentiated based on their in vitro responses. The enhanced morphogenetic response observed till the fifth subculture compared to culture initiation in both the cultivars could be due to acclimatization of the shoots to nutrient milieu, culture conditions and also due to A genomic constitution reported6.
Fig. 1—Micropropagation of *Musa* cultivars Matti and Chemmatti. (a, b)—Unripe fruit bunch of Matti, Chemmatti; (c)—Freshly prepared explant of Matti; (d)—Explant of Matti after one month of culture in MS basal medium; (e)—Multiple shoots formed after 12 weeks of culture in Matti; (f)—Multiple shoots formed after 12 weeks in Chemmatti; (g)—Shoots of Matti rooted on MS basal medium; and (h)—Hardened plantlets. [Scale bars (cm): a=18.86, b=14.65, c=0.15, d=1.6, e=1.25, f=1.25, g=1.12 and h=0.4]
Multiple shoots proliferated upon the shoot buds were divided and subcultured at 8 weeks intervals through five passages without any decline in morphology and growth of the shoots. Shoots of 4-5 cm length separated from the stock cultures so raised were transferred to MS basal media showed longitudinal growth to attain 9 cm height and simultaneously formation of up to 6 roots of 10 cm length within 4 weeks (Fig. 1g). Rooted plants (9 cm) were hardened and established at 95 and 80% rates in Matti and Chemmatti respectively (Fig. 1h). The 4-6 week old established plants subjected to cultivation in homesteads in Thiruvananthapuram and Kollam Districts, grew well and were free from abnormalities and disease. After 10 months, the micropropagated plants bloomed, producing harvestable fruits in 13 months.

Fig. 2—Effect of selected combinations of cytokinin (BAP) and auxins (IAA, IBA) on shoot formation in shoot tip cultures of Matti (A, B) and Chemmatti (C, D) primitive cultivars. [Values are mean number and height of the shoots ± SE, n=7. Each treatment for shoot culture consisted of 4 explants and repeated thrice. Observations were made after 12 weeks of culture]
No strict fertilizer schedule was followed except for the purpose. Collection of data for yield characters (Table 1). It was evident that compared to the plants conventionally raised through the suckers (15 months), the mericlones somewhat smaller in size grew fast and yielded early in Matti. No difference could be observed in growth, flowering, fruiting behaviour and shape of fruits between micropropagated plants of both the cultivars (Fig. 1a, b).

No strict fertilizer schedule was followed except the use of farmyard manure. Field data were collected after 13 months. However, as expected colour and taste between the cultivars varied, the fruit of Matti being tastier than Chemmatti. The locally held view that Chemmatti is more resistant to pests and diseases than Matti could not be confirmed, as none of the localities encountered such problems. The micropropagation method developed is expected to aid in reliable production and supply of pathogen free and disease tolerant diploid genotypes of the lesser-known taxa of the genus *Musa*. The yield characters suggested somewhat similar performance of the mericlones of Matti cultivars in all the locations selected for the purpose. Collection of data for Chemmatti is in progress.

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### Table 1—Bunch characters of Matti mericlones cultivated in the homesteads Thiruvananthapuram District.

<table>
<thead>
<tr>
<th>Place of Cultivation</th>
<th>Mean bunch weight (kg)</th>
<th>Mean number of hands per bunch</th>
<th>Mean number of fruits per bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBGRI</td>
<td>14.20 ± 0.84</td>
<td>12.00 ± 0.71</td>
<td>219 ± 33.04</td>
</tr>
<tr>
<td>Palode</td>
<td>14.00 ± 1.00</td>
<td>12.04 ± 1.50</td>
<td>180 ± 63.00</td>
</tr>
<tr>
<td>Pechiparai</td>
<td>14.20 ± 0.84</td>
<td>12.60 ± 1.10</td>
<td>194 ± 18.01</td>
</tr>
<tr>
<td>Vianur</td>
<td>14.00 ± 1.00</td>
<td>13.60 ± 1.20</td>
<td>203 ± 17.40</td>
</tr>
<tr>
<td>Kallakavilai</td>
<td>14.20 ± 0.84</td>
<td>12.80 ± 1.30</td>
<td>194 ± 22.82</td>
</tr>
</tbody>
</table>

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### References